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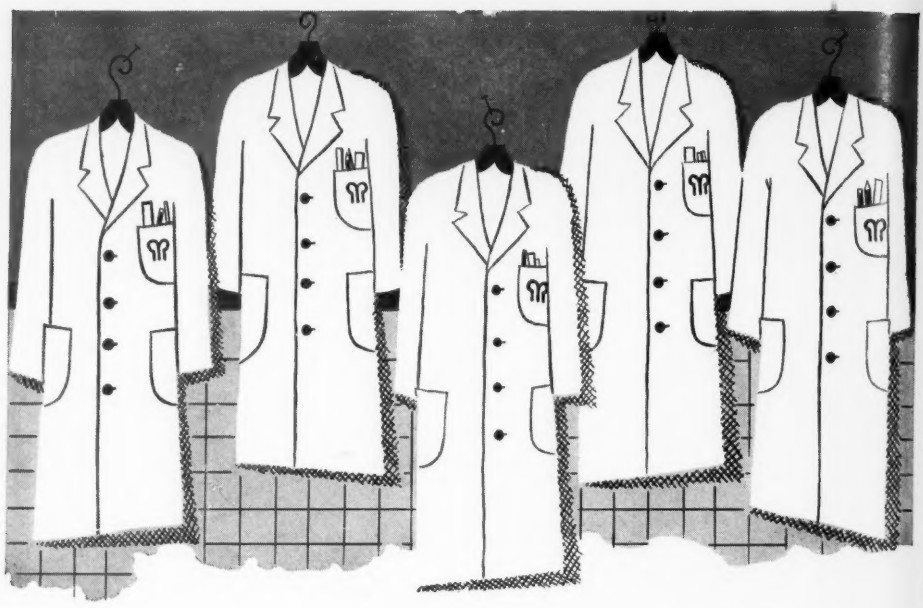

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THE EXCRETION OF 17-KETOSTEROIDS IN IDIOPATHIC HIRSUTISM

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THE observations which have been published on the excretion of urinary 17-ketosteroids in normal women show that, though fluctuations in the excretion level occur, no systematic relation can be demonstrated between these fluctuations and the different phases of the menstrual cycle (1–11). The level of ketosteroid excretion during the menstrual cycle is on the whole remarkably constant, in contrast to that of other substances of hormonal origin.

Ketosteroid excretion in hirsutism has been reported as high or very high, only when the condition is associated with the presence of a masculinizing tumor (adrenal or ovarian). In other cases of hirsutism the excretion range has been described as "within the normal range," "tending toward the male range of excretion," or as "moderately elevated" (3, 9, 12–17).

The following report deals with the 17-ketosteroid excretion of 9 patients complaining of increased growth of facial and body hair. The patients ranged in age from 21 to 30 years; 4 were single, 5 were or had been married and none had children. Menstruation in all patients was described as regular or essentially regular. The complaint of increased hair growth extended over periods varying from two to four years. Physical examination revealed no gross change in organs or other cause of this condition.

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* Agnes Lemme Schilling Fellow in Obstetrics and Gynecology.

The ketosteroids were determined in 24-hour urine specimens by an adaptation of the method of Robbie and Gibson previously described (18, 19). Specimens were collected once a week for a sufficiently long period of time to cover a complete menstrual cycle.

Figure 1 represents weekly values of ketosteroid excretion in 2 normal young women in relation to the day of the menstrual cycles. The limits of the normal female excretion range as found in this laboratory (5 to 18 milligrams per 24 hours) are indicated by horizontal broken lines. The excretion pattern is in agreement with the observation mentioned above, that there is apparently no pronounced change in the excretion level at any particular phase of the cycle.

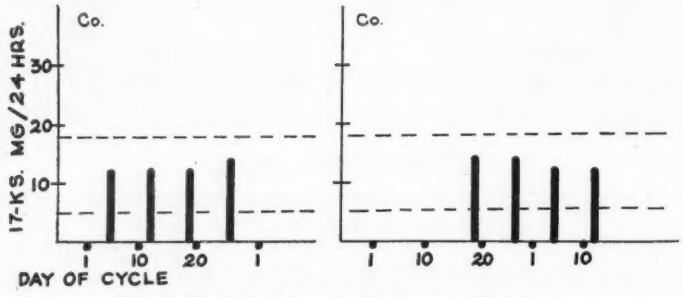

FIG. 1. Ketosteroid excretion in normal women.

Figure 2 represents in the same way the ketosteroid excretion in 9 cases of idiopathic hirsutism in which a complete record could be obtained. It is evident that a) the excretion level is in general well above the normal range, but may at times drop to normal values, b) there is a cyclic variation associated with the menstrual cycle, and c) the maximum excretion values are found at the approximate time of ovulation. The excretion levels satisfy the varying qualifications in the literature, ranging from normal to elevated, depending on the phase of the cycle during which the determination is made.

The cyclic variation pattern can be regarded as originating in one of two ways: 1) the total excretion of the adrenal cortex of these patients is subject to cyclic change, or 2) the output of the adrenal cortex does not vary appreciably but is augmented by excretion from another source which is subject to ovarian change.

If was thought useful to compare these findings with the ketosteroid excretion pattern of normal female rats in relation to the estrus cycle. To that end, total daily ketosteroid determinations were made on the urine of isolated adult virgin female albino rats with established regular estrus cycles.

The results are represented in Figure 3. It is evident that a pronounced variation of ketosteroid excretion exists, and that the maximum excretion occurs at the time of vaginal cornification, that is, at mid-estrus, corresponding to the time of ovulation in woman. The excretion pattern of these normal female rats is therefore similar, not to that of normal women, but to that of the hirsute patients.

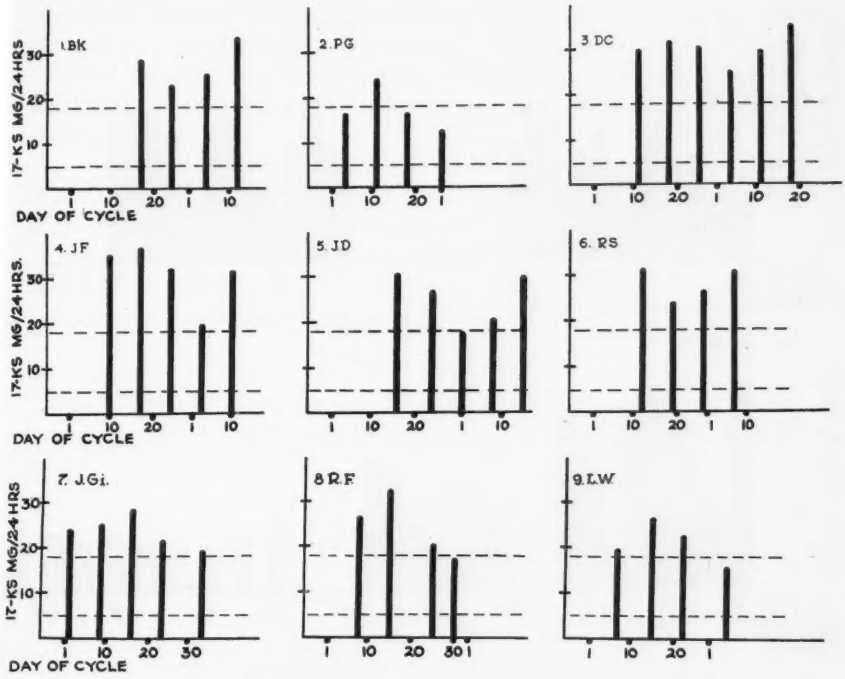

FIG. 2. Ketosteroid excretion in idiopathic hirsutism.

After ovariectomy of the rat the cyclic variation of the excretion level ceased and the level dropped to minimal values. After adrenalectomy of an otherwise intact rat, the ketosteroid excretion dropped to below measurable levels.

This pattern is evidently the chemical expression of the changes in the adrenal gland previously described by Andersen and Kennedy and by others (20, 21) who observed that the weight of the adrenal cortex of the female rat varies with the estrus cycle, reaching its highest value at estrus, and that in spayed animals the increase in weight can be brought about by injections of estrogen. This change in the adrenal cortex at the time of

estrus can be ascribed to stimulation of this organ, directly or indirectly, by the animal's estrogens.

From the similarity of the excretion patterns the conclusion can be tentatively advanced that the increased ketosteroid excretion in idiopathic hirsutism is likewise the result of a stimulation of the adrenal cortex by ovarian hormones and that the condition of these patients is due to a

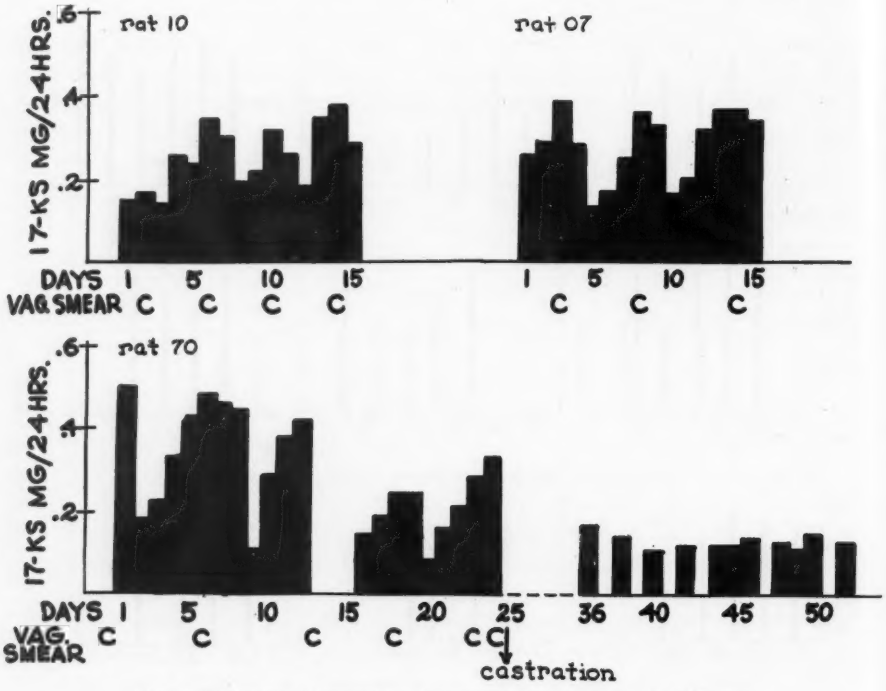

FIG. 3. Ketosteroid excretion in normal female rats. (C indicates a vaginal smear showing cornified cells.)

sensitivity of the adrenal cortex which is absent or imperceptibly small in normal women.

It should however be borne in mind that it may not be the cyclic variation of the ketosteroid excretion of the hirsute patient which requires an explanation but rather the relative constancy of the excretion during the menstrual cycle of the normal woman. It is possible that in the latter a stimulation of the adrenal cortex is balanced by a simultaneous inhibition of the adrenotropic activity of the pituitary gland. The possibility of such an inhibition seems indicated by observations of Ross and Hamblen

(22–24) of a temporary rise of ketosteroid levels after cessation of ovarian activity. Lack of data on adrenotropic hormone output in relation to the phases of the menstrual cycle does not make further speculation on this point profitable.

SUMMARY

Ketosteroid excretion levels in 9 cases of idiopathic hirsutism, measured at weekly intervals during complete menstrual cycles, show a cyclic variation with a maximum excretion at the approximate time of ovulation, in contrast to the relative constancy of excretion in normal women. The maximum levels are invariably well above the upper limits of the normal excretion range.

A similar cyclic excretion pattern with a maximum at the time of estrus is observed in normal female rats in which variations in weight of the adrenal cortex with the estrus cycle were known to occur.

The conclusion is tentatively advanced that the condition of the hirsute patient is connected with a sensitivity of the adrenal cortex to ovarian hormones which is absent in normal women.

Acknowledgment

The assistance of members of the medical profession in providing the diagnoses and in making suitable specimens available is gratefully acknowledged.

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THE ANTITHYROID ACTION OF 5-IODOTHIOURACIL, 6-METHYL-5-IODOTHIOURACIL, THIOCYTOSINE AND (Ca) 4-n-PROPYL-6-OXYPYRIMIDYL-2-MERCAPTOACETIC ACID*

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From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts, and from the Department of Medicine, University of Washington, Seattle, Washington

AN interest in the antithyroid activity of iodothiouracil and thiocytosine was expressed in an earlier report (1) of observations upon various goitrogenic agents, but neither compound was available.

It appeared possible that iodination of thiouracil might increase the concentration of the compound in the thyroid, due to the avidity of the gland for iodine. Such a selective action would render it unnecessary to expose the other tissues to as much thiouracil as is usually the case. It was thought that even though the compound did not act in this manner it might possess an advantage over thiouracil, either in possessing a stronger antithyroid action or a less toxic one. Interest in the compound persisted in spite of the fact that the antithyroid action of two iodinated aminobenzoic acids depended solely upon the effects of the iodine which they contained (1).

Dr. H. W. Barrett and associates (2) recently synthesized 5-iodo-2-thiouracil and 6-methyl-5-iodo-2-thiouracil and he and Dr. F. X. Gassner very kindly afforded us the opportunity to study these compounds.

Having studied sulfur derivatives of uracil and thymine we were interested in investigating thiocytosine.[1] It was thought that the variations in the intracellular distribution of the three natural pyrimidines might produce significantly different effects.

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* Most of the data were presented at the Annual Meeting of the Association for the Study of Internal Secretions, Chicago, June, 19, 1948.

† Present address: Department of Medicine, University of Washington, Seattle.

[1] We are very grateful to Dr. Augustus Gibson of Merck and Co., Rahway, N. J., for our supply of thiocytosine and to Dr. Ernst Oppenheimer for the (Ca) 4-n-propyl-6-oxypyrimidyl-2-mercaptoacetic acid.

We also conducted a few brief studies with (Ca) 4-n-propyl-6-oxy-pyrimidyl-2-mercaptoacetic acid,[1] which for convenience we refer to as 2-(acetic acid) propylthiouracil.

The formulas of three of these compounds are shown in Figure 1.

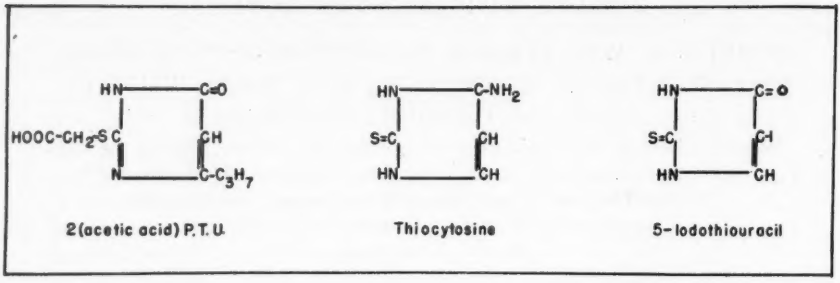

FIG. 1.

5-Iodo-2-Thiouracil

(a) *Effect upon thyroid size and concentration of non-radioiodine in rats.*

Barrett *et al.* (2) found that 5-iodo-2-thiouracil injected subcutaneously in rats was approximately one-half to one-third as effective as 2-thiouracil, judged by goitrogenesis or decreased iodine in the thyroid. When iodothiouracil was fed at a 50 per cent higher level, on a molar basis, than was thiouracil, it was found that the former was about one-half as effective as thiouracil in decreasing the (total) iodine content of the thyroid gland and less than 50 per cent as effective in goitrogenesis. When equimolar quantities of potassium iodide and thiouracil were given, the degree of goitrogenesis was greater than in the group which received iodothiouracil, and the iodine concentration in the thyroid was less (3).

(b) *Effect upon concentration of radioiodine in the thyroid of rats.*

Two experiments were conducted in rats, using radioactive iodine (I^{131}), to test the effect of iodothiouracil upon the uptake and release of the isotope by the thyroid. Its effects were compared with that of an equivalent amount of iodide alone, of thiouracil alone, and the two in combination. All of the rats used were male, Sprague-Dawley strain, and were aged approximately 5 months. Seven rats served as controls and there were 4 or 5 in each of the other groups. Each was fasted for fifteen hours and received 50 microcuries of radioiodine in 1 cc. of saline subcutaneously at the time indicated in the following paragraphs.

In the first experiment the antithyroid compound was administered two

hours before radioiodine, whereas in the second the order of administration was reversed. Each of the antithyroid compounds was administered intragastrically in 5 cc. of water. Each rat in the groups receiving iodothiouracil was given 50 mg. The ones in the groups given potassium iodide received the same amount of iodine as is present in 50 mg. of iodothiouracil. Likewise, the ones given thiouracil alone received the same amount

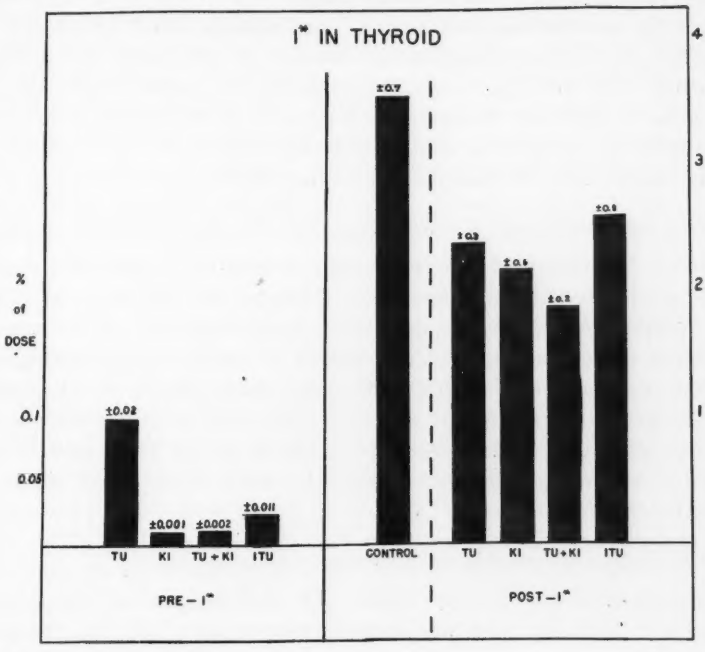

FIG. 2. The groups designated as pre-I* received the antithyroid compounds two hours before radioiodine, whereas the ones listed as post-I* were given the isotope two hours before the antithyroid compounds. The standard deviation from the mean is indicated.

as is present in 50 mg. of iodothiouracil. All rats were killed four hours after receiving radioiodine. The thyroid gland was removed, weighed quickly, ashed with potassium hyroxide and the percentage of the administered dose of radioiodine which was present in the thyroid was determined.

The results of the two experiments are presented in Figure 2. It may be observed that when any of the compounds was administered two hours before the radioiodine, the amount of the latter concentrating in the thyroid was markedly lowered. The antagonism was greater in each of the

groups receiving iodine than in the group to which thiouracil alone was given. It was slightly greater in the ones to which were administered potassium iodide than in the ones given iodothiouracil. It is difficult to know just how much of the antagonizing effect of iodothiouracil is to be attributed to the compound as such, how much to iodide and thiouracil which may have been liberated from iodothiouracil, and how much to various combinations of these compounds.

When the antithyroid compounds were administered two hours after the radioiodine larger quantities of isotopes accumulated in the thyroid than when they were given two hours before the tracer substance. However, some antagonism was exhibited by each of the compounds in each experiment. There was less difference in the degree of effects of the compounds when they were administered after the isotope.

(c) *Effect upon discharge of radioiodine from the thyroid of normal subjects.*

Doctors Astwood and Stanley kindly conducted studies with iodothiouracil similar to the ones they used in testing the antithyroid effect of many compounds (4). The assay depends upon modification of a predictable pattern of accumulation of radioactive iodine by the thyroid gland as detected by serial counts with an externally placed Geiger-Müller counter. As compared with thiouracil the action of iodothiouracil was delayed, requiring six hours to be manifested. Dr. Astwood suggested that the delayed reaction might be associated with a slow liberation of iodine from the iodinated thiouracil.

(d) *Distribution of iodothiouracil throughout the body.*

Studies conducted to determine the distribution of iodothiouracil throughout the body were unsatisfactory because of the failure of iodothiouracil to give a satisfactory color reaction with Grote's reagent.

(e) *Effect in patients with thyrotoxicosis.*

Twenty-nine unselected patients with thyrotoxicosis were given 40 courses of therapy with iodothiouracil. Twelve of the patients were hospitalized while therapy was conducted. White blood cell counts were performed upon these individuals from one to two times weekly. The other patients were ambulatory, and were observed at weekly intervals; white blood cell counts were conducted in these whenever any infection was present.

From 60 to 300 mg. was given daily in equally divided doses at approximately eight-hour intervals; the usual dose was 150 mg. per day.

The response varied considerably; some patients with severe thyrotoxicosis had a complete remission within ten days, whereas others never had

a complete recovery (see Table 1). Of 15 subjects with a maximal response in ten days, the average decrease in basal metabolic rate per day was approximately 4 per cent; of 21 with a maximal response in fifteen days, the average decrease was 3 per cent. Means and associates (5) found that with iodide therapy alone the average fall in metabolic rate was 3.6 points per day. The individuals who had not experienced a complete remission within fifteen days never had one, although some of them were treated for several additional weeks. Eleven patients never became completely free of thyrotoxicosis, although each subject showed some improvement. None of the subjects who became euthyroid experienced a relapse under continued therapy, but in a few individuals with incomplete responses the thyrotoxicosis became less well controlled, although the dosage was increased. In no instance was there a complete relapse while the patient was continuing therapy. However, following cessation of therapy a relapse was apparent within one or two weeks. Nine patients had two or more courses of therapy In three, the response was not as good with the second course as with the first, but in the others there was no essential difference.

The response in the clinical manifestations of thyrotoxicosis was in keeping with the changes in basal metabolic rate. The thyroid gland generally remained the same size or became smaller.

No definite ill-effect from therapy was observed in any case. One individual while receiving therapy developed generalized aching, malaise and sore throat. The white blood cell count was found to be 2,600, with no change in the differential blood count. The white blood count returned to normal without interruption of therapy. In view of these findings we are inclined not to attribute the illness to a toxic action of the drug.

The response to from 100 to 200 mg. daily seemed to be as good as with 200 to 300 mg. The maintenance dose appeared to be between 50 and 100 mg. daily.

Five patients were subjected to thyroidectomy following preparation with iodothiouracil. In 4 the remission was not complete at the time of operation, 3 had had severe thyrotoxicosis, and coronary heart disease was present in another. However, the operative and postoperative course of each was uneventful. Less difficulty was encountered in removing the gland than is ordinarily the case after several weeks' preparation with the customary doses of thiouracil and Lugol's solution. There was not much bleeding or friability of the gland; there was only slightly more vascularity of the glands than in nontoxic goiters. Relatively little change in the pulse rate or blood pressure during the operation or in the pulse rate and temperature following it, was noted. In each of the 5 cases the thyroid gland was found to be well involuted histologically, as illustrated in Figures 3 and 4.

TABLE 1. PATIENTS TREATED WITH IODOTHIOURACIL.

Patient No.	B. M. R. %			Maximal total daily dosage (mg.)
	Initial	Maximal response	Days required	
#103	+28	+ 9	9	100
#112	+25	0	9	100
	+39	− 8	8	100
	+25	+11	9	150
#113	+74	+20	6	150
#114	+24	+ 8	6	150
#115	+29	− 1	9	100
	+32	+10	14	150
#116	+29	+14	7	100
#117	+38	+14	24	100
#118	+50	+18	7	150
	+45	+12	20	150
#121	+26	+ 7	12	150
#122	+41	+27	15	150
	+31	+23	28	150
#123	+30	+11	7	100
	+27	+17	18	150
#125	+60	+ 1	14	150
#126	+59	+ 3	10	150
	+60	+26	28	200
#127	+25	+ 7	7	150
	+17	+ 8	15	90
#128	+45	+15	13	150
#129	+50	+11	7	100
	+54	+ 7	30	150

TABLE 1—*Continued*

Patient No.	B. M. R. %			Maximal total daily dosage (mg.)
	Initial	Maximal response	Days required	
#130	+61	+23	18	200
#131	+14	+ 1	8	90
#134	+55	+21	7	300
#135	+18	+ 9	15	150
#137	+55	+12	43	150
#138	+13	+ 9	18	150
	+35	+18	21	150
#139	+83	+10	9	150
	+41	+36	9	300
#140	+70	+22	68	300
#141	+60	+38	7	250
#143	+34	+21	7	150
#145	+69	+21	33	200
#146	+29	+10	35	200
#147	+24	+ 9	15	150

Some of the responses to therapy can be illustrated by consideration of a few individual cases.

Case 1. In Figure 5 is presented the course of one patient as illustrated by change in the basal metabolic rate. The patient was a fireman, aged 38, who had manifestations of thyrotoxicosis for three months. The thyroid was diffusely enlarged, weighing approximately 30 Gm. The basal metabolic rate was plus 26 per cent. With 150 mg. of iodothiouracil daily for twelve days the metabolic rate decreased to plus 7 per cent and the manifestations of thyrotoxicosis disappeared. After 100 mg. daily, divided in two doses, for seven additional days the rate was plus 5 per cent. Then upon giving only 50 mg. daily in one dose for two weeks, the metabolic rate increased to plus 22 per cent; after 50 mg. twice daily for twelve days, the rate was plus 9; after 30 mg. thrice daily for two weeks it was plus 6, and it was maintained within a normal range for the next four weeks. However,

within four weeks after cessation of therapy the metabolic rate was plus 46 per cent. Thus, whereas 50 or 60 mg. daily failed to control the thyrotoxicosis, 90 mg. daily was successful.

Case 2. In Figure 6 is presented the response to therapy, as exemplified by the basal metabolic rate, of a housewife, aged 41, who had had manifestations of thyrotoxicosis for six months. The thyroid gland was enlarged diffusely to approximately 60 Gm. With 50 mg. of iodothiouracil twice daily for nine days the basal metabolic rate decreased from plus 25 per cent to 0. After 50 mg. daily, in one dose, for twelve days the rate was plus

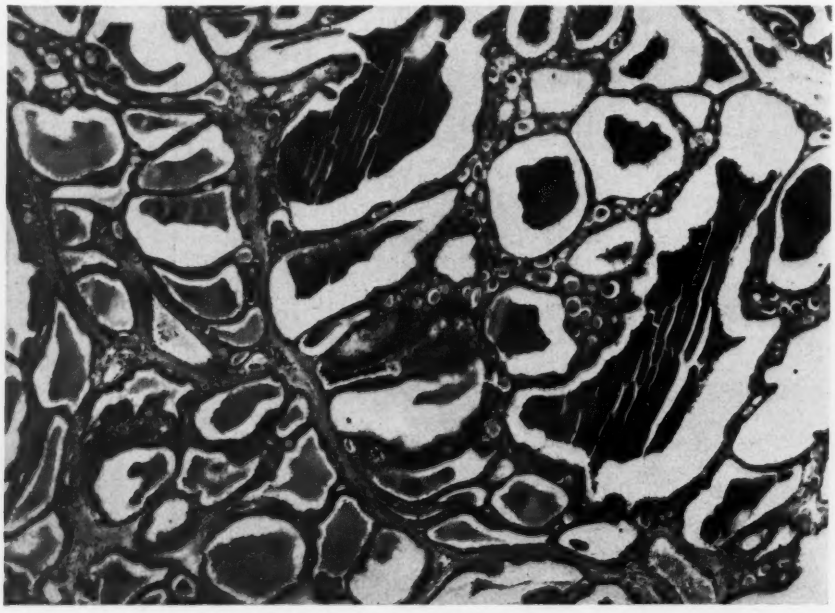

FIG. 3. Photomicrograph of thyroid (×100) of patient whose metabolic response to therapy is shown in Figure 8.

8 per cent. Therapy then was discontinued. Three months later the basal rate was plus 39 per cent; after 50 mg. twice daily for eight days it was minus 8. Therapy was again discontinued. Six weeks later the metabolic rate was plus 40 per cent, and after therapy for nine additional days it was plus 11 per cent. Therefore, this patient experienced three separate and complete remissions of thyrotoxicosis. Each of the first two remissions occurred very rapidly but the third one required a longer interval.

Case 3. In Figure 7 is illustrated the response to therapy of a fireman, aged 35, who had had thyrotoxicosis for three years. After 50 mg. of propylthiouracil three times daily for sixty days, the basal metabolic rate decreased from plus 89 per cent to plus 25; after 100 mg. three times daily for thirty days, the rate was plus 40. After an interval of eight months with no therapy the metabolic rate was plus 59 per cent. Then, following 50 mg.

of iodothiouracil three times daily for ten days, the rate was plus 3 per cent. The dosage was reduced to 50 mg. twice daily and after seven days the rate was plus 5. Therapy was then discontinued for five weeks. At the end of this interval the metabolic rate was plus 60. Propylthiouracil was given in doses of 100 mg. thrice daily and after nine days the metabolic rate was unchanged. Following this, iodothiouracil was given for five weeks, beginning with 30 mg. three times daily and gradually increasing the dosage to 50 mg. four times daily. The basal metabolic rate did not become normal again, but the patient experienced an almost complete remission of the thyrotoxicosis, clinically. The response was much slower than with the first course of therapy.

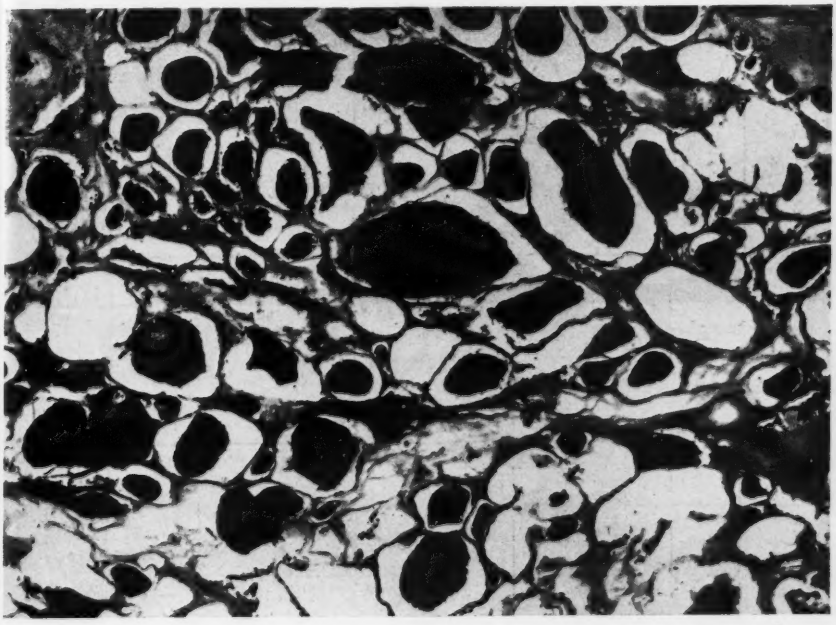

FIG. 4. Photomicrograph of thyroid (×100) of a patient, aged 71, with moderately severe thyrotoxicosis and enlargement of the thyroid to approximately 35 Gm. Iodothiouracil was given for forty-five days in total daily doses beginning with 150 mg. and gradually increased to 250 mg. The patient became euthyroid. A subtotal thyroidectomy was performed and 35 Gm. of tissue was removed. The operative and postoperative courses were smooth. Very little bleeding or friability of the gland was observed. Histologic examination revealed marked involution throughout.

Case 4. In Figure 8 is illustrated the course of a patient who was treated for seventy days with doses which were larger than those given to most of the other patients, but without experiencing a complete remission of his thyrotoxicosis. He was a laborer, aged 50, who had severe thyrotoxicosis and pituitarigenic oculopathy. The thyroid was enlarged diffusely to approximately 45 Gm.; the basal metabolic rate was plus 70 per cent. After prolonged therapy with iodothiouracil, he experienced a distinct clinical improve-

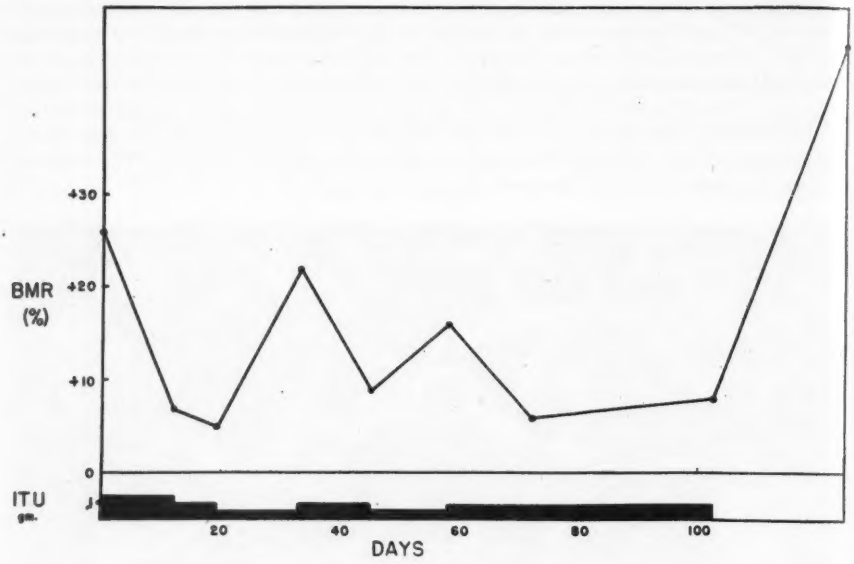

FIGURE 5.

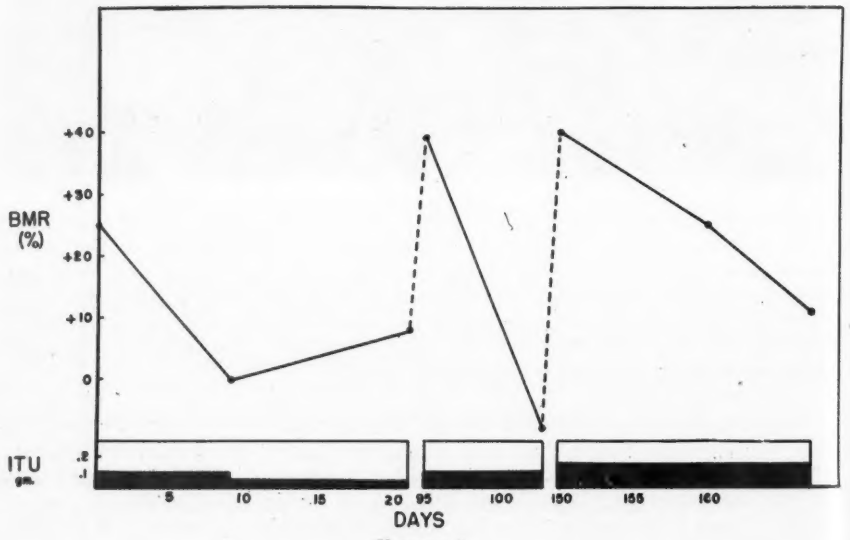

FIGURE 6.

ment, but he always remained thyrotoxic. The iodothiouracil was administered for seventy days and was the only antithyroid therapy which he received preoperatively. He had a smooth operative and postoperative course. His pulse never rose above 100 and there was very little bleeding or friability. The gland weighed 43 Gm. As shown by the photograph in Figure 3, the tissue showed relatively good involution.

The data that have been presented indicate that iodothiouracil can in some cases produce a rapid and complete remission of thyrotoxicosis. In Figure 9 comparisons are made of the maximal fall in basal metabolic rate

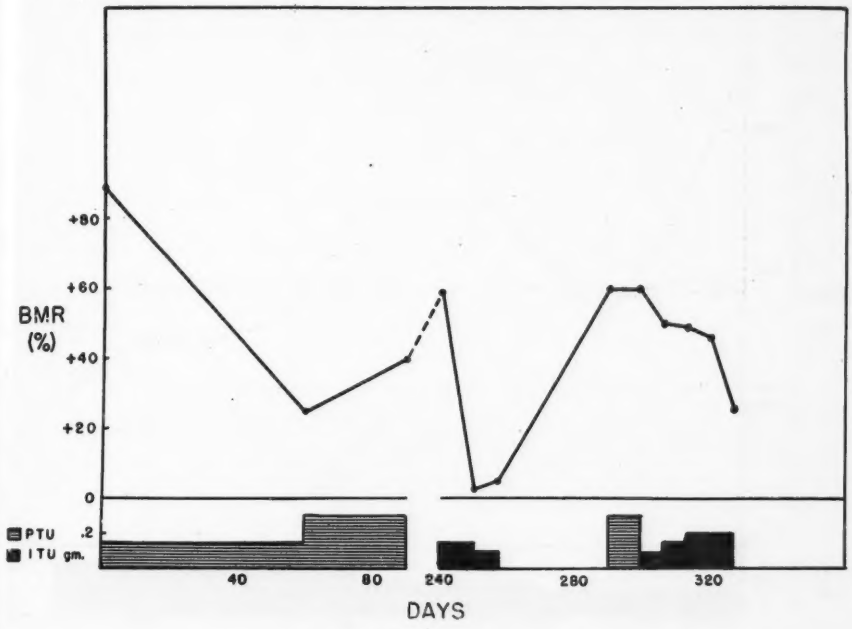

FIGURE 7.

in response to thiouracil, iodide and iodothiouracil. The maximal responses to iodothiouracil are not as good as the ones to thiouracil but better than the ones to iodine. The results of Maxwell, Gunter and Schwarz (7) using standard doses of thiouracil and iodide show essentially the same distribution as iodothiouracil when plotted in the same manner as the ones in Figure 9. However, this type of plotting does not take into consideration the dosage of the compounds used or the interval required to produce the response. Maxwell and associates state, "our experience is that thiouracil and iodine given concurrently from the commencement of treatment would in most cases reduce such a basal metabolic rate (plus 55 per cent)

to normal in 15 to 20 days." These investigators used as initial doses 600 mg. daily of thiouracil and 5 minims thrice daily of Lugol's solution. In certain of our cases 90 mg. of iodothiouracil daily was the maximum quantity used to produce a remission. In no instance was there good evidence that a daily dosage greater than 150 mg. was superior to the latter. It is to be emphasized that with 150 mg. of iodothiouracil only approximately

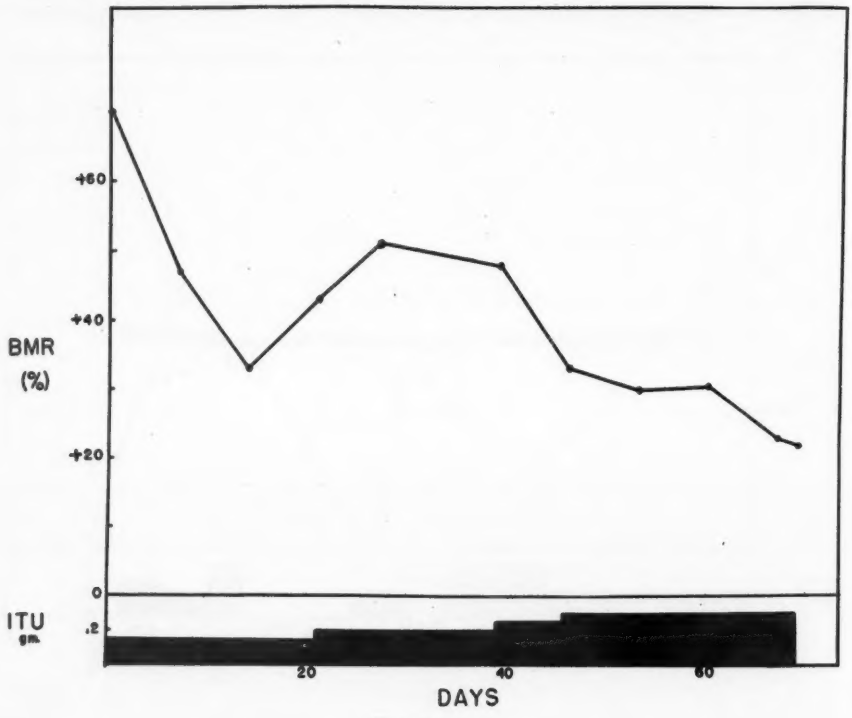

FIGURE 8.

75 mg. of thiouracil is being received. Within fifteen days this quantity of thiouracil has a neglibible effect upon a patient with definite thyrotoxicosis. The quantity of iodine in 150 mg. of iodothiouracil is approximately 75 mg. which is the amount in two or three drops of a saturated solution of potassium iodide. Although much larger doses of iodine are usually given, the studies of Thompson (8) and Lerman (9) indicate that rarely is a dose in excess of 6 mg. necessary.

Whether iodide and thiouracil given in doses comparable to the respective amounts used in the iodothiouracil therapy are as effective as is the latter can only be determined by conducting this specific experiment.

Our results indicate that the responses obtained with iodothiouracil are much more like those of iodide than of thiouracil. Among the similarities may be mentioned: (a) variations in response by different people, or even by the same individual who received several courses of therapy, (b) very rapid and complete response in certain individuals, (c) most of the changes

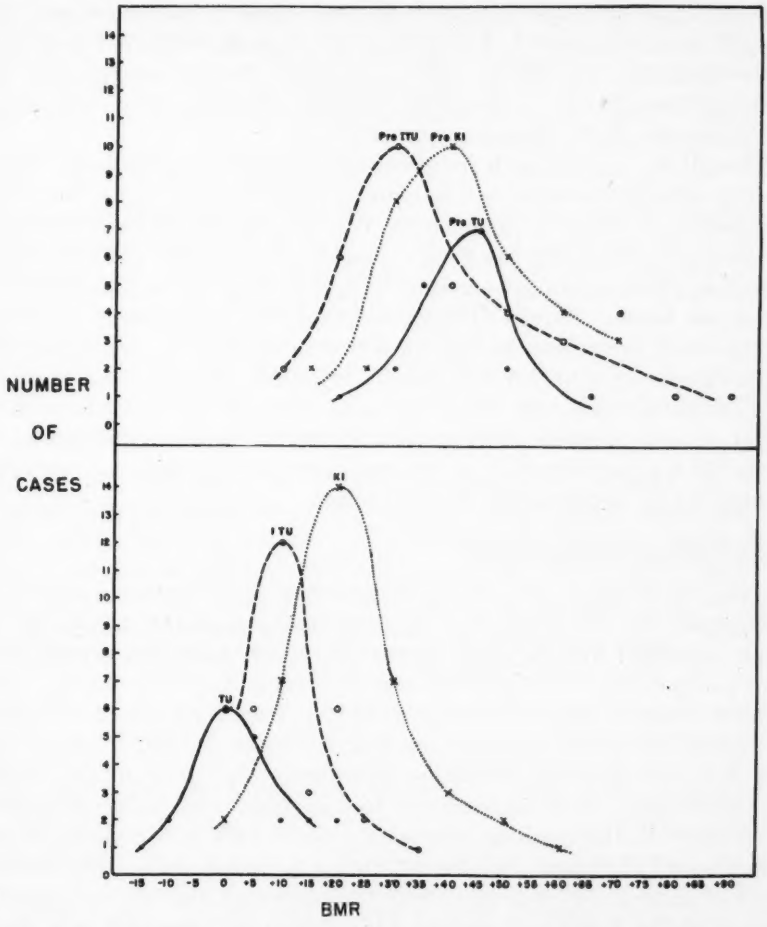

FIG. 9. In the top graph is shown the frequency distribution range of basal metabolic rates before therapy and in the bottom one is presented the response following iodine, thiouracil, or iodothiouracil. The data showing the responses to iodine and thiouracil were taken from Moore and collaborators (6). It is apparent that a greater proportion of patients had a fall in basal metabolic rate to a normal range following iodothiouracil than after potassium iodide.

produced occurred in the first seven to fifteen days of therapy, (d) the effects of 100 to 150 mg. daily were essentially as good as those from 200 to 300 mg., (e) exacerbations in thyrotoxicosis occurred upon changing from iodothiouracil to propylthiouracil, even though the same dosage of the latter had previously been effective, (f) the operative and postoperative responses to therapy, as well as the histologic appearance of the thyroid, simulated those of iodide therapy more than those of thiouracil, and (g) although certain patients had normal basal metabolic rates and were free of thyrotoxicosis, the disease was much more readily precipitated by emotional disturbances in them than in individuals in whom the same clinical response had followed thiouracil.

Although the results with iodothiouracil simulate qualitatively those obtained with iodide we have the impression, but not the proof, that it is somewhat more effective than iodide. Whether the thyroid concentrates iodothiouracil sufficiently to afford a more constant supply of iodine than is afforded when equivalent amounts of iodine are given as potassium iodide, is not known. Barrett (10) showed that rats fed amounts of iodide and thiouracil equivalent to the constituents of each in iodothiouracil, concentrated only approximately one-half as much iodine in the thyroid as when the latter compound was given alone. Since in each instance a subnormal amount of iodine was found, it is difficult to know whether there was less of a thiouracil effect or whether some of its action was counterbalanced by the iodine which it contributed.

5-Iodo-6-Methyl-2-Thiouracil

In view of the fact that methylthiouracil exerts a somewhat stronger antithyroid action than thiouracil, hope was entertained that the iodinated compound might also be more potent. However, quite the reverse was found in 6 patients with thyrotoxicosis (*see* Table 2). Three of these subjects had complete remissions with 5-iodothiouracil both before and after the methyl derivative was administered; a relapse was permitted to develop before each course of therapy was begun. A fourth subject (#123) had essentially a complete remission to 5-iodothiouracil before and after the course with the methyl compound. Patient #125 had a complete remission to iodothiouracil, but did not receive a second course of it. The response of patient #130 was essentially the same with the two compounds. In none of the 6 patients treated with iodomethylthiouracil was there better than a slight clinical response. One would think that some of the iodine would be liberated and that at least a common iodine response would be obtained. The difference in the effects of the two iodinated compounds is of great interest, but is not clear.

TABLE 2

Compound tested	Patient No.	B. M. R. %			Total daily dosage (mg.)
		Initial	Maximal response	Days required	
5-Iodo-6-methyl-2-thiouracil	#112	+26	+37	9	100
	#115	+26	+32	19	150
	#123	+45	+27	25	150
	#125	+37	+63	36	150
	#129	+49	+54	15	100
	#130	+40	+18	28	150
(Ca)-4-n-propyl-6-oxypyrimidyl-2-mercaptoacetic acid	#125	+32	+48	47	200–400
	#127	+17	+21	28	250–400
	#135	+27	+24	8	250
	#141	+37	+60	21	250–400
	#151	+45	+29	35	300–400
Thiocytosine	#139	+60	+41	21	150

(Ca) 4-n-Propyl-6-Oxypyrimidyl-2-Mercaptoacetic Acid

This compound was used in the treatment of 5 patients, in total daily doses ranging from 200 to 400 mg. Little improvement was observed in any patient, although one was treated continuously for forty-seven days and another for thirty-five days. No toxic reactions were encountered.

Dr. Astwood kindly tested the effect of this compound upon the concentration of radioiodine in the thyroid, in the manner discussed pre-

TABLE 3

Amount of compound			Thyroid size	
Per cent solution	Mg. consumed per 100 Gm. body weight		Mg. per 100 Gm. body weight	
Compound	Thiouracil	Thiocytosine	Thiouracil	Thiocytosine
.0001	0.3	0.4	7.8	8.6
.0005	1.6	1.5	8.1	8.2
.001	2.4	3.2	6.7	7.7
.005	14.4	13	8.7	8.3
.01	23.8	30	11.4	9.5
.1	200	244	17.2	17.9

viously. He found that 100 mg. showed no effect and, therefore, by this test its activity was less than 25 per cent of a comparable amount of thiouracil.

Thiocytosine

Dr. Astwood also tested this compound, in the manner previously described, and found that its activity was less than 25 per cent of that of thiouracil.

The goitrogenic effect of thiocytosine in rats was found to be similar to that of thiouracil (Table 3).

One patient with severe thyrotoxicosis was given 50 mg. of thiocytosine three times daily for twenty-one days. Although the basal metabolic rate decreased from plus 60 to plus 41 per cent there was no definite clinical improvement. No additional supply of the compound could be obtained for testing other patients.

SUMMARY AND CONCLUSIONS

Forty courses of therapy with 5-iodo-2-thiouracil were administered to 29 patients with thyrotoxicosis. The responses simulated qualitatively those following iodide therapy more than those which thiouracil produces. In 15 patients the maximal response occurred within ten days and in 21, within fifteen days. The individuals who had not become euthyroid within fifteen days did not attain this state with further continuation of such therapy. Five patients who received iodothiouracil in preparation for subtotal thyroidectomy experienced a smooth operative and postoperative course. The thyroid gland revealed a relatively good degree of involution.

Iodothiouracil inhibited the uptake of radioiodine by the thyroid of rats slightly more than did an equimolar dose of thiouracil, but less than an equivalent amount of iodine given as potassium iodide.

5-Iodo-6-methyl-2-thiouracil was used in the treatment of 6 thyrotoxic patients. It was much less effective than was its nonmethylated analogue.

(Ca)-4-n-propyl-6-oxypyrimidyl-2-mercaptoacetic acid was used in the treatment of 5 patients with thyrotoxicosis and thiocytosine was employed in 1 subject. No patient received significant benefit from either drug. Each compound was distinctly less active than was thiouracil in antagonizing the uptake of radioiodine in the thyroid by normal subjects who had received tracer doses of the isotopes several hours previously.

Thiocytosine exhibited essentially the same amount of goitrogenesis in rats as did thiouracil.

None of the patients experienced a toxic reaction to any of the compounds studied.

The results with 5-iodothiouracil are sufficiently encouraging to warrant further testing of this compound and other similar iodinated ones in the preparation of thyrotoxic patients for thyroidectomy. Comparisons of the effects of equimolar quantities of iodide and thiouracil should also be made.

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RETARDED ABSORPTION OF PELLETS OF PROTAMINE-ZINC INSULIN

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AFTER Parkes and Young (1939(1)), Mark and Biskind (1940(2)), Cutting, Morton and Cohn (1941(3)) and Parkes (1942(4)) proved the inefficacy of subcutaneous implants of insulin, either pure or with cholesterol, this experimental path was apparently abandoned. McCullagh and Lewis (1942(5)) reported prolonged effects with subcutaneous implantations of tablets of insulin-cholesterol in 4 dogs and of silver tubes filled with crystalline insulin and open at one end, in 5 others. All dogs were normal. A definite decrease in the blood sugar was evident for a period of twenty-one days. Potent insulin was found in the removed tissue-encapsulated remnants of the implants. In spite of these discouraging results, we decided in 1943 to carry on the study of insulin implants, a therapeutic method which was giving excellent results with liposoluble hormones in clinical medicine. Our experiments in normal (Vargas, Koref and Zañartu, 1944(6)) and diabetic rabbits (Vargas, Koref and Lewin, 1948(7)) implanted with a protamine-zinc-insulin complex resulted so satisfactorily that one of us has been able to apply it in clinical practice (Vargas and Lewin, 1948(8) and Vargas, 1949(9)).

METHODS

All the experiments were carried out in normal rabbits and in rabbits made diabetic by alloxan (Dunn *et al.* 1943(10)) or alloxantin (Koref *et al.* 1944(11)). Males and females were used indifferently, their weight varying between 1,000 and 2,000 Gm. Their food was fresh grass, carrots, compressed rabbit's pellets[1] and water *ad libitum*. We used crystallized insulin from the Instituto Bacteriológico de Chile, and amorphous insulin from the Instituto Sanitas in order to make the protamine-zinc-insulin in a way similar to the commercial methods. The tablets or pellets of insulin, with or without cholesterol, were prepared directly from the crystalline or amorphous powder. Those of protamine-zinc-insulin were made from the powder, in the following way: the PZI complex obtained at pH 7.1, as in the commercial method, was filtered through a hard filter paper; the pre-

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[1] Manufactured by Williamson, Balfour & Co. and containing wheat, alfalfa, peanuts, NaCl and $CaCO_3$.

cipitate, together with the filter paper, was placed in a vacuum desiccator containing sulphuric acid and when dry, it was gathered from the filter paper and powdered in a small mortar. When the mixture with cholesterol (c.p. Pfanstiehl) was used, the proportion was 1:1.

The tablets were made in a small tableting machine. Their diameter was 6 mm. and their weight varied between 22.2 mg. and 58.6 mg. The pellets were made in a special apparatus which insured uniform compression; their diameter was 1.5 mm., their length varied between 2 and 10 mm. and they weighed between 3 and 14 mg.

The material was implanted in the subcutaneous tissue and in a few instances, in the muscle. The glycemia was determined by the Hagedorn-Jensen micromethod, usually after a period of twelve hours' fasting. The tablets and pellets, when removed from the animals, were freed from the capsule, dried and weighed. In 63 normal animals, 88 implantations were performed; and in 11 diabetic rabbits, 17 implantations. Table 1 shows

TABLE 1. DETAILS OF THE IMPLANTATIONS PERFORMED IN NORMAL AND DIABETIC RABBITS

Group	No. of animals	Number of implantations				Form
		Insulin	Insulin-chol.	PZI	PZI-chol.	
			Subcutaneous			
Normal	63	10	4	10	15	Tablets
		—	—	—	35	Pellets
			Intramuscular			
		—	—	—	3	Tablets
		—	—	—	11	Pellets
			Subcutaneous			
Diabetic	11	—	4	1	7	Pellets
			Intramuscular			
		—	—	—	5	Pellets

the details. Among the 63 implanted normal animals, glycemia was tested for in 12. In the diabetic animals, in addition to determining the amount of glycemia, tests were also made for glycosuria (Fehling's method) and acetonuria (Gerhardt's method). The body weight was determined in both groups two or three times a week.

RESULTS

Insulin alone, implanted in tablet form into normal rabbits did not avoid rapid absorption, even though it was observed that the implants, which

varied between 488 and 778 international units, did not cause death before the sixth day. However, the animals finally died in hypoglycemic shock. With all the other forms of insulin used, the majority of the nondiabetic animals survived with no change in their normal growth. The glycemia curves showed a depression during fasting, which in the cases treated with PZI or PZI-cholesterol lasted for approximately fifty days after the implantation (Fig. 1).

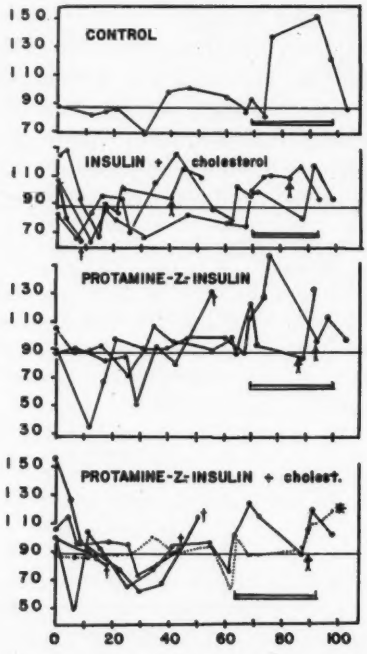

FIG. 1. *Values for blood sugar, after a 12-hour fast, in normal rabbits implanted subcutaneously with tablets of different types of insulin.*

Abscissae, time in days; ordinates, milligrams of glucose per 100 cc. of blood. The double horizontal lines in the 70–90 day area indicate that values were obtained without fasting. The implantation was made on 0 day. Arrows indicate extraction of the tablet;† spontaneous death;* the tablet was not found.

It was observed that the tablets of PZI (after 5, 91 and 96 days) and of PZI-cholesterol (after 22 and 100 days) still contained insulin, producing a lowering of blood sugar when an extract of them was injected into normal rabbits. This effect was absent in recovered pellets of insulin-cholesterol. The tablets of insulin-cholesterol have almost the same weight before and after the implantation, even increasing their weight in some

cases. This fact prevented calculation of the rate of absorption of the insulin-cholesterol implants.

If in normal rabbits there is a prolonged insulin effect, in rabbits with alloxan diabetes it is even more evident. The diabetic animals used in this series as a control lived between four and thirty-two days, whereas the diabetic animals treated with a sufficient dose, especially of the mixture PZI-cholesterol, showed a tendency towards complete normalization. It was possible to observe them for over two hundred days (Table 2). In the diabetic animals the best results were obtained with implants of 430 and 690 units per kilogram of body weight. Only animal No. 36/7 died from hypoglycemic shock, six days after the implantation of 510 units per kilogram of PZI-cholesterol. However, this animal was in a bad nutritional condition after the administration of alloxan, having lost 290 grams in seven days, and the shock occurred after a fasting period of eighteen hours. Of especial interest is animal No. 1/50 which, after presenting a very severe diabetes, received 2 implants of PZI-cholesterol (1,530 and 1,331 units, respectively) and recovered completely from its diabetes. Biopsy specimens taken from the pancreas 170 days after the first implantation and later, 861 days after the injection of alloxan and 365 days after the second implant, showed an evident damage of the beta cells of the islands of Langerhans in the first histologic specimen, but signs of regeneration and frank cellular recuperation in the second specimen (Gomori's stain). Figure 2 shows the evolution of the diabetes in animal No. 1/50. Figure 3, as an example of insufficient treatment, shows the evolution of the disease in animal 1/30, in which on the nineteenth day the implant was partially eliminated due to suppuration. Animal No. 1/30 is a control for animal No. 1/50. Both had a similar degree of diabetes, both received similar treatment with alloxan and PZI implants, at the same time. During the preimplant period (thirteen days) both required about five injections of PZI (daily dose 10 to 16 I.U.). The high requirement of insulin of No. 1/30 is again demonstrated when, on the thirty-fifth day after the implantation, insulin injections were needed again. From then on, twenty-three injections were given in increasing doses, the last being 80 I.U., without control of the diabetic condition.

Absorption

The calculation of the absorption of the insulin was based on the difference in weight of the pellets before the implantation and after their recovery. The method was similar to that used by other writers for mixtures of liposoluble hormones with cholesterol.

The implants were removed after a certain number of days; if one animal received several implants at a time, these were removed at different

Table 2. Implantations Made in Alloxan-Diabetic Rabbits

Animal No.	Sex	Weight		Alloxan 5% mg./Kg.	Implantation				Days of Observation			Results			
													Average blood sugar in mg. %		
		I. Gm.	F. Gm.		Form	Mg.	IU	IU/Kg.	Before implant	During implant**	Total	General	Pre-implant	During implant	Post-implant
					Implantation of PZI										
32/38	F	2700	2700	200	T.* Subcutan.	53	1060	450	11	14	25	insufficient		390† (382–400)	—
					Implantation of insulin and cholesterol (40%)										
32/36	M	1520	2350	100	T. Subcutan.	29.4	353	200	24	8	91	insufficient	285 (273–298)	—	—
					T. Subcutan.	58.5	702	360		27		insufficient		275 (181–343)	—
					T. Subcutan.	108	1296	550		28		insufficient		247 (80–328)	—
40/57	F	950	1340	200	T. Subcutan.	91	1092	870	5	2	7	excessive	337	40	—
					Implantation of PZI and cholesterol (50%)										
1/14	F	2420	1920	135	T. Subcutan.	27.8	278	130	22	43	78	insufficient	384 (351–400)	270 (204–349)	
					T. Subcutan.	33.4	334	160		4		insufficient		—	—
					T. Subcutan.	56.2	562	250		9		insufficient		—	—
32/15	M	2520	1800	198	T. Subcutan.	29.8	298	140	7	3	11	insufficient	390	385	
					T. Subcutan.	68.4	684	350		1		insufficient		350	—
32/91	M	1800	2200	200	T. Subcutan.	28.8	288	140	10	61	71	insufficient	228	166 (121–193)	
36/7	F	1600	1460	187	P. Intramusc.	67.1	671	510	7	7	14	excessive	242	26	—
36/22	F	1950	1700	200	P. Intramusc.	100.0	1000	600	8	80	88	good	252	121 (88–235)	—
36/34	F	2300	2620	185‡	P. Intramusc.	149.2	1492	690	7	22	84	good	202	60 (33–77)	—
1/50	F	2300	2620	43	P. Intramusc.	153.0	1530	680		48		good	316 (313–320)	263 (196–326)	237 (166–304)
					P. Subcutan.	153.0	1331	430	14	227	861	good		158 (70–245)	96 (70–140)
1/30	M	2300	1825	54 41 200	P. Intramusc.	199.0	1990	820#	18	122	153	insufficient	338 (317–374)	356 (334–400)	—

* T = Tablets. P = Pellets.
** Period during which the implant remained in the body.
† The glycemia was determined five days after the implantation. The figures in parentheses correspond to minimum and maximum results.
‡ Alloxantin 2.5%.
# Many pellets (about 50%) eliminated after the nineteenth day of the implant.

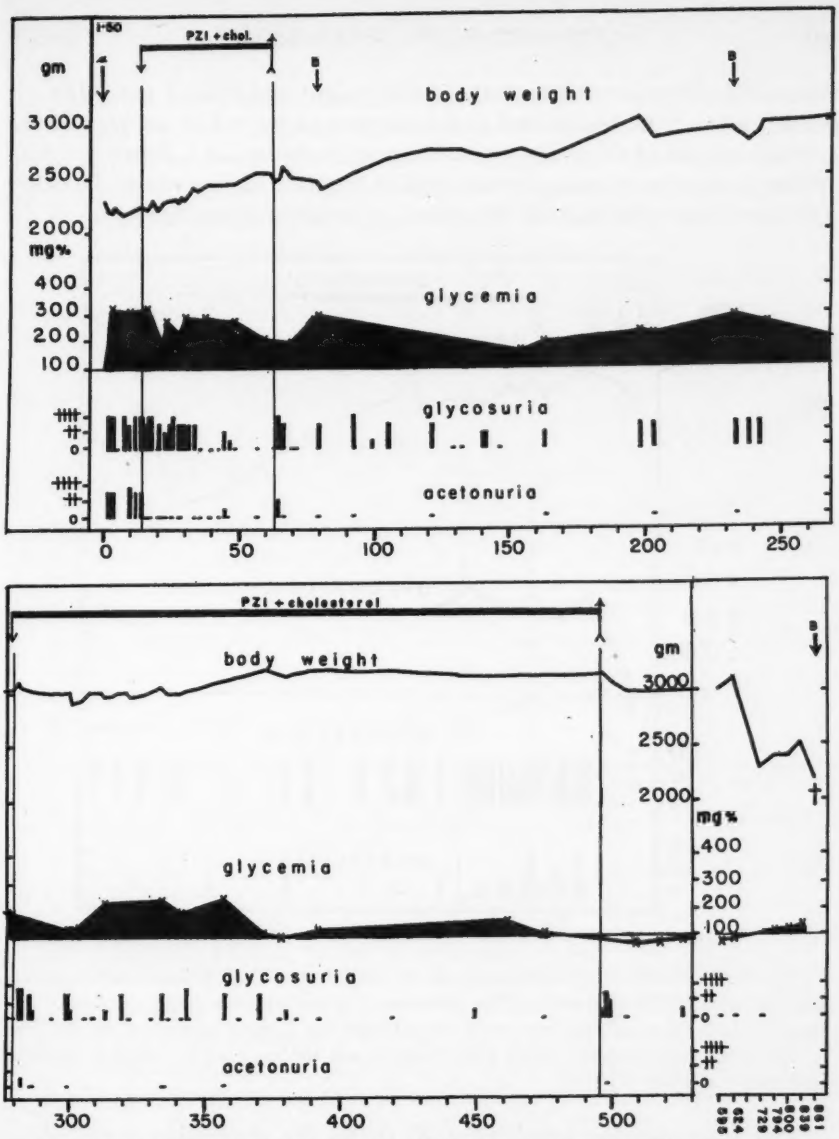

FIG. 2. *Rabbit No. 1/50,* with alloxanic diabetes treated with two implants of PZI-cholesterol. *Above:* first implant. *Below:* second implant. During the first pre-implant period of fourteen days, the animal received 7 subcutaneous injections of 10 to 20 I.U. of PZI (Lilly); after the first implant, these injections were suspended. Note that glycemia decreased and glycosuria-acetonuria disappeared during the period of the first implantation; complete normalization was attained during the second implantation and persisted almost indefinitely.

Abscissae, time in days; arrow "a," alloxan injection; arrow "B," biopsies of pancreas; arrows joined by horizontal line denote periods of implantation (the implant was removed at the end of the line).

* Period during which the implant remained in the body.
† The glycemia was determined five days after the implantation. The figures in parentheses correspond to minimum and maximum results.
‡ Alloxan 2%.
§ Many pellets (about 50%) eliminated after the nineteenth day of the implant.

times. The difference between the initial weight and that of the pellet (or tablet) taken from the animal and dried, was expressed in per cent of the original weight of the pellet (percentage of reabsorption). From the data on the percentage of reabsorption, and on the days during which the pellet remained under the skin of the animal, a graph was constructed.

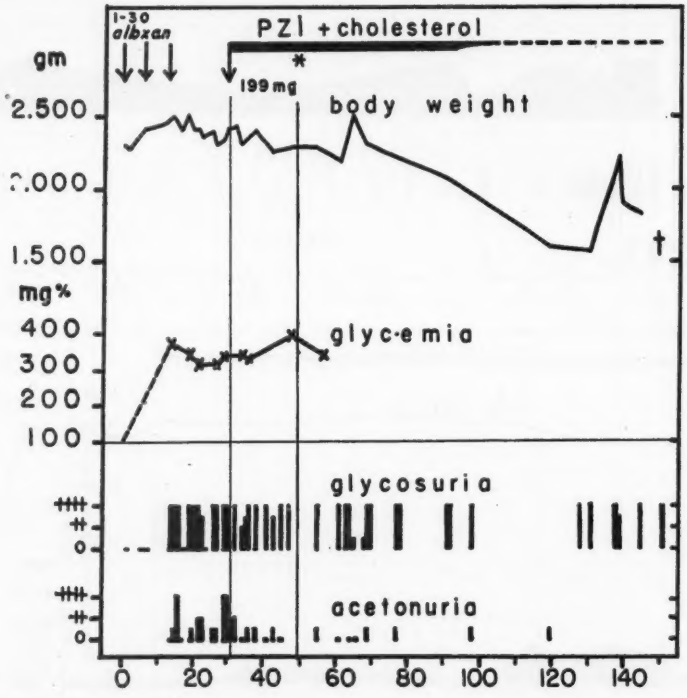

FIG. 3. *Rabbit No. 1/30,* with alloxanic diabetes treated insufficiently with only one implantation of PZI-cholesterol. The dose was divided into two parts, implanted into separate legs. The material was partly eliminated by abscess formation on one side. Abscissae, ordinates and other abbreviations are the same as in Figure 2. Asterisk indicates expulsion of pellet.

The accompanying graph (Fig. 4) shows the absorption curve of the PZI-cholesterol implants. In relation to the percentage of absorption, the shape of the pellet does not seem to have any influence upon the rapidity of absorption of the substance. It is of interest to remember that we are working with a protamine-insulin complex, of which solubility in water is fairly low and of which the reabsorption is even more retarded by the reaction of the tissues (pH 7.0–7.1). The cholesterol only facilitates the

compression and permits a better conservation of the shape of the pellet. Although we have not studied the curve of absorption of PZI without cholesterol, the few points shown in the graphs indicate the same rate of daily absorption as for the PZI-cholesterol. This permits us to deduce that with this mixture the absorptions of insulin and cholesterol are probably proportional. The absorption is completed about the one hundred and thirtieth day. In general, we can state that the implant is conserved in the body for at least one hunrded days, its absorption being slightly more

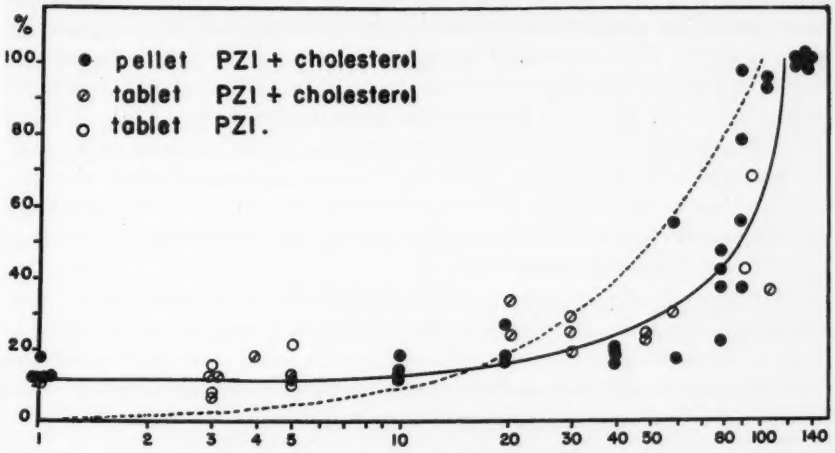

FIG. 4. *Curve of absorption of tablets and pellets of protamine-zinc-insulin and PZI-cholesterol (50 per cent) in normal rabbits.*

Abscissae, time in days in logarithmic scale; ordinates, total percentage of absorption. The dotted curve corresponds to 1 per cent daily absorption.

rapid in the last twenty or thirty days. This is probably due to the disintegration of the implant which gives it a greater surface of absorption. A daily absorption of approximately 1 per cent of the total quantity implanted is a fairly accurate practical calculation. Therefore, knowing the daily insulin requirement, it is easy to calculate the amount of insulin which should be implanted (*i.e.*, 20 units daily calls for an implant of 2,000 units).

DISCUSSION

Our results are contrary to those of Mark and Biskind (2). They stated that pellets of protamine plus zinc-insulin, in a proportion of 4:1, influenced the diabetes of pancreatectomized dogs for not more than 48–100 hours. However, their preparation of this mixture was different from ours because they simply mixed the protamine with the zinc-insulin without obtaining

previously the complex at the required pH. Their mixture made into pellets is absorbed as rapidly as insulin alone, being freely soluble in the tissue fluids. That is why pellets of this mixture in larger doses (261 and 1,012 units) produced hypoglycemic shock and in smaller doses (43–88 units) produced only a transitory lowering of the blood sugar, which lasted 48–100 hours. Furthermore, they stated that pellets prepared from the dry powdered commercial PZI complex, as described here by us, were inactive. They could not explain why the complex was altered by desiccation, in spite of the fact that the redissolution of one of these pellets, prepared from the commercial product, caused a lowering of the glycemia when injected. This shows that actually there was no alteration of the PZI and that the dry powder conserved its activity. The implants produced no change in the glycemia because the doses used were too small. When a few milligrams are implanted,[1] the absorption of the complex is so slow, (as we have already shown), that it is not enough to influence the glycemia either in the normal or in the diabetic animal. It is the same as if an insufficient dose of insulin was injected. In this way Mark and Biskind failed to ascertain the useful dose.

Cutting *et al.* (3), implanted pancreatectomized dogs with pellets of insulin-cholesterol and obtained an effect upon the glycemia which lasted as long as thirteen days. These results agree with ours, since we have shown that cholesterol prolongs the absorption of pure insulin although it is not capable of avoiding a relatively fast absorption.[2]

SUMMARY

1. It has been shown experimentally in normal rabbits that protamine-zinc-insulin is the only type of insulin useful for subcutaneous implantations. This protamine-zinc-insulin compound, either in tablets or in pellets, alone or mixed with 50 per cent cholesterol, is absorbed so slowly that its lowering effect on the blood sugar lasts at least as long as fifty days.

2. Subcutaneous or intramuscular implants of protamine-zinc-insulin complex with 50 per cent of cholesterol in alloxan diabetic rabbits, when used in doses of from 430 to 690 units per kilogram of body weight, control the diabetes and metabolic changes for approximately one hundred days.

3. The results with this type of implant enable us to calculate a daily absorption of approximately 1 per cent of the amount implanted. This

[1] The authors do not state the dose used in their experiments but it must have been the same as that used with the other material they implanted, *i.e.*, 4.0 mg.

[2] We take this opportunity to thank Drs. Jorge Lewin and Juan Villalobos for their help in the preparation of the PZI-cholesterol pellets and in controlling some of the implants.

estimate is appropriate for clinical application, and we recommend it for further study in human diabetics.

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THE BEHAVIOR OF LABELED IODOCASEIN IN HUMAN MYXEDEMA*

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STUDIES of the distribution and transformation of calorigenic, iodine-containing substances have been handicapped by the fact that pharmacologic doses of material were usually required in order to obtain a concentration of iodine in tissue or body fluids sufficiently high to be measured satisfactorily by current chemical methods. The availability of radioiodine (I^{131}) has made it possible to reinvestigate the metabolism of calorigenic iodine compounds in animals and man under physiologic conditions. Radioiodine can be incorporated into calorigenic materials so as to obtain biologically active compounds with high specific activity. This not only permits the use of physiologic amounts of material, but also permits determination of the distribution of the material and its derivatives with greater accuracy and sensitivity than was previously possible. In addition, radioiodine and substances containing it can, to some extent at least, be observed in vivo by means of a Geiger counter directed at various parts of the body.

The present study represents an initial effort to explore the distribution and transformation of calorigenic compounds labeled with radioiodine. Iodocasein, an artificial iodoprotein, was chosen for this study because it was felt that its synthesis from casein and radioactive iodine might be accomplished with less difficulty and less radiation hazard than that of other materials. Iodocasein has been proved to be actively calorigenic in animals and man (1, 2) and essentially similar in its biologic behavior to other iodoproteins which have previously been employed in man (3, 4).

A myxedematous patient having little or no thyroid function consented to be the subject of the study. This patient was chosen in order to minimize or avoid the possible complications which might result from the turn-

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over of labeled iodide (or perhaps of other catabolic by-products of the material given) by the thyroid gland. Considering the remarkable capacity of the thyroid to accumulate iodine and to synthesize it into thyroid hormone, it was anticipated that the presence of the thyroid gland might obscure the primary metabolic events following the administration of exogenous thyroid-like material which we wished to study. Once the extrathyroidal behavior of such materials has been evaluated, the effect of the intact thyroid upon them may well become a subject for future investigation.

MATERIALS AND METHODS

The plan of the study was to obtain several observations on the distribution and fate of labeled iodocasein in a patient with myxedema at two metabolic levels: 1) while hypothyroid, before treatment, and 2) while euthyroid, after adequate treatment of the myxedema with nonradioactive iodocasein. As control data, exactly similar observations were made with labeled sodium iodide in amounts comparable to that of the iodine contained in the iodocasein. Both labeled materials were administered by mouth.

1. Report of case. The patient selected was a married, white woman, aged 45 years, who had postoperative, postirradiation myxedema. In April, 1947, partial thyroidectomy with resection of the left lobe had been performed for nodular goiter without hyperthyroidism. The pathologic examination revealed a solid adenocarcinoma, grade 2 (Broders' method) with the extra-adenomatous portion of the thyroid showing marked thyroiditis and regenerative hyperplasia. After thyroidectomy, she had been given a course of radium therapy. The patient returned on September 9, 1947, with the signs and symptoms of myxedema, including characteristic changes of voice, facies and skin as well as slowed tendon reflexes. The body weight was 77.05 Kg. The basal metabolic rates were −26 and −29 per cent, and the concentration of cholesterol was 420 mg. per 100 cc. of serum. A tracer dose (for diagnostic purposes) of 100 microcuries of I^{131} as sodium iodide with 100 micrograms of stable sodium iodide as carrier revealed a total excretion in the urine of 86.9 per cent in seventy-two hours. This high urinary excretion is typical of myxedema (5).

The patient was transferred to the metabolism unit and placed on a 1,400 calorie repetitive diet, which contained a low but constant amount of iodine. The iodine content was not determined directly, however. The preparations used in this investigation and the order of their administration are given in Table 1. Clinically the patient was followed by daily observations of body weight, basal pulse and blood pressure, and determination at least twice weekly of basal metabolic rate and serum cholesterol.

After the initial radioiodine studies during myxedema were completed, a daily dose of 60 mg. of nonradioactive iodocasein was administered. Fourteen days after the initiation of this therapy, subjective and objective evidence of improvement was obvious. After thirty-seven days of treatment, the signs and symptoms of myxedema were completely alleviated. The basal metabolic rate had risen from −29 per cent to 0, the cholesterol had declined to 167 mg. per 100 cc. of serum, and the weight from 77.05 Kg.

TABLE 1. CHEMICAL AND ISOTOPIC CHARACTERIZATION OF THE MATERIALS ADMINISTERED DURING THE INVESTIGATION

Date given	Preparation no.	Material	Dose, mg.	Iodine content, mg.	I^{131} content, microcuries
9-18-47	1	NaI* +carrier	0.1	0.085	100
9-27-47	2	NaI* +carrier	3.62	3.0	175
10- 3-47	3	Radioactive iodocasein (P_M)†	70.0	3.5	170
10- 9-47 to 11-16-47	4	Nonradioactive iodocasein (P_C)†	60.0	3.7	
11- 4-47	5	NaI* +carrier	3.62	3.0	155
11-11-47	6	Radioactive iodocasein (P_N)†	63.0	3.4	230

* Radioactive.

† The abbreviations P_M, P_C and P_N refer to specific preparations described in detail elsewhere (8).

to 70.93 Kg. (Fig. 1). At this point, the patient was judged to be in a euthyroid state, and the studies with inorganic radioiodide and radioiodocasein were repeated. At the end of the study, which required fifty-seven days for completion, the patient was dismissed with instructions to take 1½ grains (0.1 Gm.) of desiccated thyroid[1] daily. During a year of observation which followed, she has remained euthyroid and in good health on this dose.

[1] The desiccated thyroid employed was Parke, Davis & Company strong uncoated thyroid, which is assayed by the manufacturer as 50 per cent stronger than U.S.P. thyroid.

2. Laboratory methods. A) *Radioactive compounds.*—The preparation of labeled iodide for clinical use has been previously described (6). In the present study, enough NaI^{127} was added in order for the iodine content of the labeled iodide doses to equal the iodine content of the dose of labeled iodocasein, which was approximately 3.0 mg. (Table 1).

Iodocasein and radioiodocasein were prepared by a modification of the method of Reineke and Turner (7). The preparations used contained between 5 and 6 per cent of iodine by weight; 25 to 35 per cent of the iodine

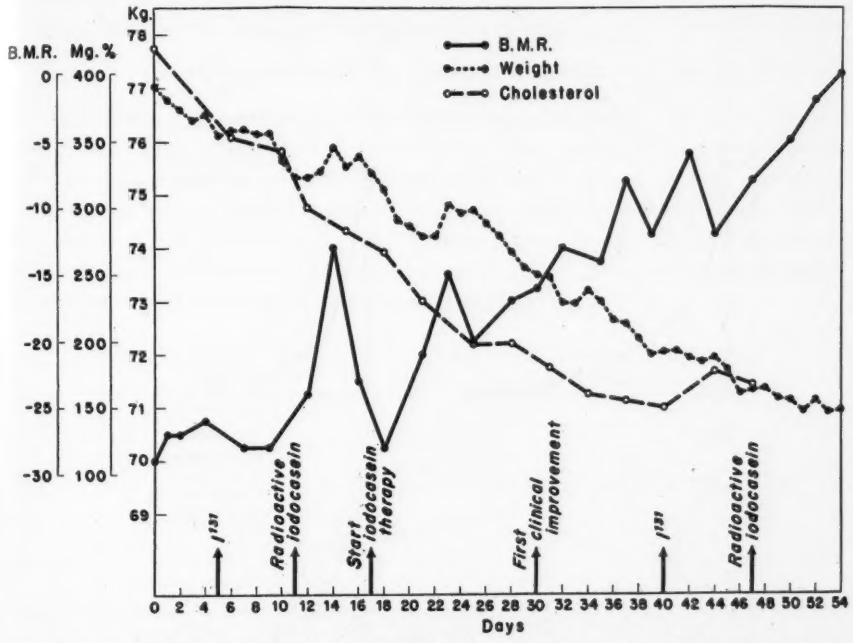

FIG. 1. The clinical course of the patient during therapy with iodocasein as indicated by changes in basal metabolism, body weight, and serum cholesterol.

present was combined as thyroxine. The technical procedures involved in preparation of iodocasein and radioiodocasein are described in a separate paper, in which characteristics and analyses of the preparations used in this study are described in detail (8). The biologic potency of the iodocasein preparations as determined by bio-assay in tadpoles of *Xenopus laevis* was compared with that of desiccated thyroid and a physiologic daily dose for the artificial hormone was estimated to be 60 mg. (9).

B) *Analyses of radioactivity.*—Urine was collected and analyzed as described elsewhere (6). Stools were collected separately over a five-day

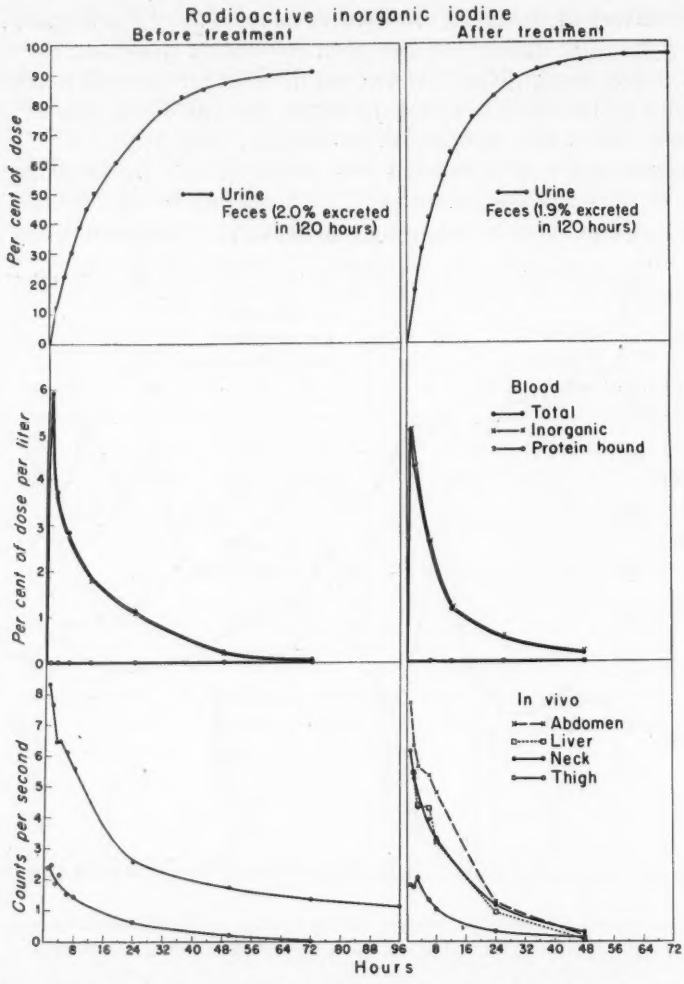

FIG. 2. The behavior of orally administered labeled iodide during myxedema and euthyroidism as indicated by measurement of urinary and fecal excretion, blood levels, and in vivo observations.

period, and if only small amounts of radioactivity were present, the stools were pooled for analyses. The Kendall (10) open ashing method of preparing the stools was used. In place of titration, however, 0.5 cc. of the final mixture was counted in wet form with a beta ray (thin mica window) counter. Determinations of radioactivity in the feces were checked by measurements with a gamma ray counter. Measurements of radioactivity

in blood were made according to the procedures used by McConahey and his associates (11). Total serum I^{131}, serum precipitable (protein-bound) I^{131} and nonprecipitable (largely inorganic iodide) I^{131} were estimated on serial samples after the ingestion of each tracer dose.

In vivo measurements of radioactivity were made serially over the thigh, neck, liver and epigastrium. The method for these procedures is described in detail elsewhere (12, 13).

C) *Chemical analyses.*—Chemical determinations of the plasma protein-bound iodine were kindly performed for us by Dr. Douglas Riggs, Boston, Massachusetts, to whom we are greatly indebted.

RESULTS

1. Control date (the behavior of labeled inorganic iodide).—Figure 2 shows the distribution of radioiodine in the urine, feces, serum and various parts of the body, after the oral administration of a dose of radioactive inorganic iodide, before treatment when the patient was hypothyroid and after treatment when she was euthyroid. The results of the chemical determinations of the protein-bound iodine at various intervals after administration of the radioactive inorganic iodide are listed in Table 2.

TABLE 2. PROTEIN-BOUND IODINE AT VARIOUS INTERVALS FOLLOWING ADMINISTRATION OF RADIOACTIVE INORGANIC IODIDE

Hours	Hypothyroid	Euthyroid
	Micrograms per 100 cc. of plasma	
0	1.8	9.8
6	2.3	
12	2.0	12.0

When the patient was hypothyroid, 91.1 per cent of the inorganic radioiodide administered was excreted in the urine in seventy-two hours and 93.9 per cent in 142 hours. Over a period of five days, a total of 2 per cent of the dose appeared in the feces. Essentially the same distribution of I^{131} in urine and feces was noted when the patient was euthyroid. The curves of total serum radioactivity were essentially similar at both metabolic levels, with the exception that radioiodine disappeared somewhat more rapidly from the serum during the euthyroid state. Fractionation of the serum disclosed that in the hypothyroid state substantially all of the serum I^{131} was in the form of iodide until forty-eight hours after the administra-

tion of the labeled iodide. After forty-eight hours, however, when the total radioactivity was very small, I^{131} appeared in the precipitable fraction, which constituted more than half of the total serum I^{131}. When labeled inorganic radioiodide was ingested in the euthyroid state, all of the serum radioactivity remained in nonprecipitable form. The chemical determination of protein-bound iodine did not reveal significant changes at various times after the administration of radioactive inorganic iodide either in the hypothyroid or in the euthyroid state. In vivo measurements were substantially the same in both myxedema and euthyroidism. No accumu-

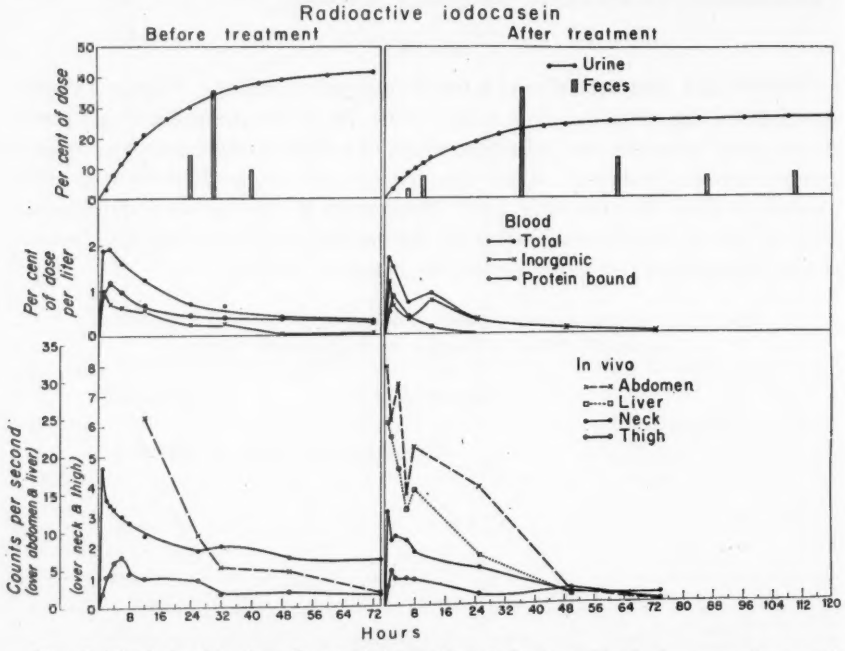

FIG. 3. The behavior of orally administered labeled iodocasein during myxedema and euthyroidism, as indicated by measurements of urinary and fecal excretion, blood levels, and in vivo observations.

lation of iodide was noted in any of the tissues, and in all cases the labeled iodide disappeared rapidly and uniformly from the various sites measured.

2. Behavior of labeled iodocasein.—Organic I^{131} (radioiodocasein) behaved quite differently from inorganic radioiodide, as comparison of Figure 3 with Figure 2 indicates. The I^{131} excreted in the urine during myxedema totaled 41.9 per cent of the radioactivity of the labeled dose in seventy-two hours, 43.6 per cent in 144 hours and 44.2 per cent in 216 hours. Within forty hours, 59.4 per cent of the dose had appeared in the

feces. During the euthyroid state, excretion of I^{131} in the urine amounted to 24.1 per cent in forty-eight hours, 25.7 per cent in seventy-two hours and 26.6 per cent in 144 hours. Over a 110-hour period, 51.7 per cent was excreted in the feces. In both experiments, most of the fecal radioactivity was found in a single stool excreted twenty-eight to thirty-two hours after administration of the radioactive iodocasein.

The maximal concentration of radioactivity observed in serum was very much less (about a third) after administration of radioiodocasein than after administration of inorganic radioiodide. At both metabolic levels, peaks of radioactivity in serum were reached in one hour, after which there was a progressive decline. In the hypothyroid state detectable amounts of radio-

TABLE 3. PROTEIN-BOUND IODINE AT VARIOUS INTERVALS FOLLOWING ADMINISTRATION OF RADIOACTIVE IODOCASEIN

Hours	Hypothyroid	Euthyroid
	Micrograms per 100 cc. of plasma	
0	1.5	12.4
6	4.6	20.6
12	3.8	12.9

iodine were present 144 hours after administration of the dose of iodocasein, but in the euthyroid state, very little was found after forty-eight hours. In the myxedematous state, one hour after administration of radioiodocasein, the radioiodine in the blood was found equally in the precipitable and nonprecipitable fractions. At forty-eight hours, 92 per cent was protein bound and at 144 hours all of the radioiodine in the blood appeared in protein-bound form. In the euthyroid state, however, 30.5 per cent of the radioiodine was protein bound in one hour, 57.3 per cent in two hours, 17.7 per cent in twelve hours and none in twenty-four hours.

Significant differences were noted by chemical determinations of the protein-bound iodine (Table 3) at varying intervals after ingestion of the labeled material. When the patient was myxedematous, administration of radioactive iodocasein resulted in values two to three times as great as the initial level of 1.5 micrograms per 100 cc. At the euthyroid level, similar studies disclosed almost a doubling of the protein-bound iodine six hours after ingestion of the dose, from an initial level of 12.4 micrograms per 100 cc.

After the administration of iodocasein, in vivo measurements did not reveal significant uptake of radioactivity in the thyroid area, either be-

fore or after control of the myxedema. However, the measurements over the abdomen, and particularly over the liver, were greatly increased within the first few hours, but decreased rapidly within twenty-four hours.

COMMENT

This study was planned so that the behavior of organically bound iodine could be compared with the behavior of the same amount of inorganic iodide at two levels of metabolism. These comparisons were made because of the possibility that the distribution and fate, not only of organically bound iodine, but also of inorganic iodide, might be different in hypothyroidism than in euthyroidism. In order to evaluate this factor so far as iodocasein was concerned it became necessary to study the distribution of iodocasein after the patient had been brought to equilibrium at a euthyroid level with nonradioactive iodocasein.

This program of study led to one finding with respect to inorganic iodide which for the moment remains unexplained. Approximately the same small proportion of the dose (4 per cent) remained unaccounted for in urine and feces in both hypothyroid and euthyroid studies with inorganic radioiodide. This small quantity undoubtedly reflects analytic errors, but nonetheless it probably reflects also a residue which is presumably distributed diffusely in other tissues of the body, as reported elsewhere (6). The thesis that it is not concentrated significantly receives some support from our failure to demonstrate localization by in vivo observations. Nevertheless, during myxedema a small quantity of I^{131} in the serum (0.04 per cent of the dose per liter) was found in precipitable form. No such precipitable fraction appeared in serum following administration of inorganic iodide when the patient was euthyroid. This finding could be interpreted as representing a minute and delayed synthesis of radioiodide into organic radioiodine compounds by tissues other than the thyroid (the so-called extra-thyroidal synthesis of thyroid hormone) which may be operative in myxedema and suppressed in the euthyroid state. The finding could also be attributed to synthesis of organic radioiodine in minute amounts by some residual thyroid tissue too small in amount to be detected by our in vivo methods, or it might represent an analytic artefact.

In other respects, the behavior of inorganic radioiodide at the two metabolic levels was consistent with the known effects of thyroid hormone on hemodynamics. Iodide disappeared more rapidly from the blood at a euthyroid level than at a myxedematous level (largely owing to a faster renal excretion rate), as indicated by more rapid appearance of radioiodine in urine, more rapid disappearance from blood and more rapid disappearance from peripheral tissues measured in vivo.

The metabolism of organic iodine, as contained in radioiodocasein, is

however, much more complex than that of iodide. One must consider (14, 15) that soon after ingestion, some of the material might theoretically be absorbed intact; part might be broken up by digestive enzymes, yielding smaller fragments such as thyroxine and diiodotyrosine, as well as other amino acids and various peptide aggregates containing iodine, which would be more readily absorbed; lastly, depending on the completeness of digestion, some inorganic iodide might be liberated and absorbed. In addition to such complexities, the role of the liver must be considered, since it has been demonstrated that iodinated compounds may be secreted in the bile and also that liberation of inorganic iodide from such compounds may occur after passage through the liver. Such material as is excreted in this manner may be wholly or partly reabsorbed. Finally, some of the iodocasein or its fragments may be excreted from the digestive tract, either as unabsorbed material or as material that has been secreted into the bowel. The over-all picture may thus be a complicated one, consisting of digestion, absorption, intermediary metabolism, secretion and reabsorption.

The fractionation of the serum radioiodine into precipitable and nonprecipitable fractions at various times after ingestion affords a general view of the sum of all such processes, but obviously cannot delineate any one of them. Any radioiodocasein absorbed intact as well as absorbed fragments of iodocasein, including diiodotyrosine and thyroxine, would in all probability appear in the precipitable or protein-bound fraction of serum, whereas liberated iodine in the form of inorganic iodide would appear in the nonprecipitable fraction. The analysis indicated in Figure 2 shows that iodocasein has been split rapidly, since within one hour an appreciable proportion of the serum radioiodine is in the form of inorganic iodide, and the remainder in organic form. This is also borne out by the chemical analysis for protein-bound iodine (Table 2). That the liver may contribute to this breakdown is suggested by the high concentration of radioactivity over this organ during the first twenty-four hours. The slow disappearance of inorganic radioiodide from serum over the course of three days would indicate a persistent release of iodide from iodocasein or its fragments, as the further fate of this inorganic radioiodide should follow the course outlined previously by the control study.

The fecal excretion of radioiodine after administration of the labeled doses of iodocasein amounted to more than half of the ingested material. Analysis of the radioiodine in feces showed that more than 90 per cent was in protein-bound form. Again it is not possible to determine the exact nature and origin of these compounds, but it would appear reasonable to regard this excretion as the sum of the processes mentioned previously.

The most notable difference between the behavior of radioiodocasein in the hypothyroid state and its behavior in the euthyroid state appeared in

the quantity recovered in the urine; 43.6 per cent in 144 hours in the first instance compared with 26.6 per cent in the same period in the second instance. The difference between these figures in effect constitutes "lost iodine," since no clue can be found from other observations which suggests what became of it. We were unable to find any technical error which would explain the difference and at the moment we have no explanation for it. Similar studies of other subjects may be necessary to determine whether this observation has significance.

A further observation of interest has to do with the correlation of protein-bound iodine and the clinical status of this patient. After therapy with iodocasein, in amounts considered equivalent to a maintenance dose of desiccated thyroid as determined by bio-assay in tadpoles, the protein-bound iodine was 12 micrograms per 100 cc. of plasma, a "hyperthyroid" level by most standards, despite the fact that the patient was clinically euthyroid. After four months of therapy with natural thyroprotein (0.1 Gm. of strong desiccated thyroid daily) the patient remained euthyroid clinically, and the protein-bound iodine had fallen to 4.0 micrograms per 100 cc. of plasma, a "euthyroid" level.

No exact explanation for this behavior can be given, inasmuch as one human assay does not permit deductions concerning the relative calorigenic potency of iodocasein versus desiccated thyroid. The following possibilities could be considered. Since the amount of organic iodine and of thyroxine iodine was three or more times greater in the dose of iodocasein than in that of desiccated thyroid, the equal metabolic effect could be due to poorer utilization of iodocasein than of desiccated thyroid. It will be recalled that more than half of the radioiodine of radioiodocasein was eliminated in the feces. Assuming that this represented half of the thyroxine radioiodine also, and (as seems likely) that the thyroxine content of iodocasein alone determines its calorigenic action, then the isocalorigenic effect could be partly explained on the grounds of inefficient absorption. To account now for the disparity in protein-bound iodine is not difficult, since iodocasein contains organic iodine in three or more times greater amounts in the form of nonthyroxine compounds than does desiccated thyroid. In the latter, the nonthyroxine iodine is essentially all in the form of diiodotyrosine, a substance that may be rapidly metabolized. In iodocasein, however, in addition to diiodotyrosine, there may exist other iodinated amino acids. The behavior of these could be quite similar to the behavior of such organic iodine compounds as gallbladder dyes (priodax) which, as is well known, are without calorigenic effect but are capable of elevating the level of protein-bound iodine for long periods.

SUMMARY AND CONCLUSIONS

1. Iodocasein has been shown to correct the signs and symptoms of human myxedema. Although our observations do not permit an accurate appraisal of the relative potency of iodocasein as compared with natural thyroprotein, a considerably higher level of protein-bound iodine in blood was associated with maintenance of euthyroidism by iodocasein than by desiccated thyroid.

2. Comparative studies with labeled inorganic iodide (NaI^{131}) during myxedema and euthyroidism indicate that no demonstrable localization occurs in the athyrotic subject, nearly all of the iodide being accounted for in urine or feces. Furthermore, no transformation of iodide was observed, except possibly in the myxedematous state, in which small amounts of radioactivity were found in the protein-bound fraction of serum.

3. Comparative studies with labeled organic iodine (radioactive iodocasein) during myxedema and euthyroidism disclosed concentration of radioactivity over the gastro-intestinal tract and liver, high fecal and low urinary excretion. As indicated by blood levels of radioactivity and by fractionation studies, iodocasein was rapidly absorbed. Rapid and continuous degradation of the material was indicated by the early appearance and persistence of a significant labeled inorganic iodide fraction in serum.

4. Both iodide and iodocasein were disposed of more rapidly while the subject was in a euthyroid state than in a hypothyroid state.

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COMPARATIVE VALUE AND ACCURACY OF MEASUREMENTS OF URINARY I^{131} BY BETA AND BY GAMMA RAY COUNTING*

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In our investigation of I^{131} to induce myxedema in euthyroid patients with angina pectoris and congestive failure (1) and in many studies of the effect of I^{131} in thyrotoxicosis and thyroid cancer, the retention of this agent in the thyroid has been estimated indirectly by measuring the urinary excretion (2–6) over a period of three days. The fraction retained in the body is assumed to reside mainly in the thyroid gland. In evaluating the accuracy of measurement of I^{131} in the urine, we observed a considerable decrease in counts per minute when equal amounts of I^{131} were added to urine as compared to distilled water. The purpose of this report is to evaluate this source of error and to describe the comparative advantages of two methods for measurement of I^{131} activity in urine.

METHODS

A. Measurement of Urinary I^{131} by Counting Beta Rays

A 1.0 cc. aliquot of urine containing I^{131} is pipetted into cleansed, weighed aluminum cups 3.1 cm. in diameter and 0.4 cm. high. Approximately 0.1 mg. NaI (1 drop 0.2 per cent NaI) and 2–3 mg. gelatin (1 drop 10 per cent gelatin) are added. Samples are evaporated to dryness at 37–40° C., and the time required for 4,096 counts determined, using an end-window Geiger-Mueller tube (mica window thickness of 3.2 mg./cm.^{2}) in constant positional relationship to the cup. Counts per minute are calculated, and net counts per minute obtained by subtracting background counts. A standard is counted between every fourth sample to check the constancy of the Geiger-Mueller tube and scaling circuit as well as to afford the basis for calculating the amount of I^{131} in the sample.

Duplicability

One cc. of water containing I^{131} was pipetted into each of 10 cups; approximately 0.1 mg. NaI and 2–3 mg. gelatin were added, and the samples

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dried at 37–40° C. The final dry weights varied from 1.8 to 2.9 mg. (average 2.4 mg.). The net counts per minute (Table 1) varied from 8,150 to 8,740; the mean was 8,420. The standard deviation was 207.

TABLE 1. DUPLICABILITY OF COUNTS/MINUTE OBTAINED IN 10 IDENTICALLY PREPARED SAMPLES

Cup No.	Cup weight (Gm.)	Wt. of cup and dry residue (Gm.)	Wt. of dry residue (mg.)	Corrected cts./min.* $\times 10^3$
1	1.1237	1.1255	1.8	8.74
2	1.1481	1.1501	2.0	8.62
3	1.1440	1.1464	2.4	8.15
4	1.1089	1.1113	2.4	8.52
5	1.1238	1.1262	2.4	8.30
6	1.0896	1.0921	2.5	8.52
7	1.1292	1.1317	2.5	8.50
8	1.1292	1.1317	2.5	8.20
9	1.1323	1.1349	2.6	8.15
10	1.1420	1.1449	2.9	8.50

* Corrected counts per minute = counts per minute less background counts per minute. In this experiment background = 18 counts per minute.

Effect of pH

Three 10.0 cc. aliquots of an I^{131} solution were diluted to 1,000 cc. with distilled water and adjusted to pH 6.0, 7.0 and 8.6 respectively. Cups were prepared by the above described technique. Samples at pH 6.0 showed approximately 35 per cent less counts than samples at pH 8.6 (Table 2).

TABLE 2. EFFECT OF pH OF URINE ON MEASURED I^{131} ACTIVITY

Sample	pH	Corrected counts per minute $\times 10^3$	Average	Per cent alkaline samples
1	8.6	5.65	5.58	100
2	8.6	5.63		
3	8.6	5.45		
4	7.0	5.26	5.09	91
5	7.0	5.05		
6	7.0	4.92		
7	6.0	3.62		
8	6.0	3.58	3.60	64.5

Studies at more alkaline pH were not desirable because of digestion of the aluminum cup.

Coincidence error

In counting high activity samples, considerable error is introduced due to coincidence loss. This is illustrated in the curve established by plotting the daily counts against the calculated curve of decay (Fig. 1). It is our

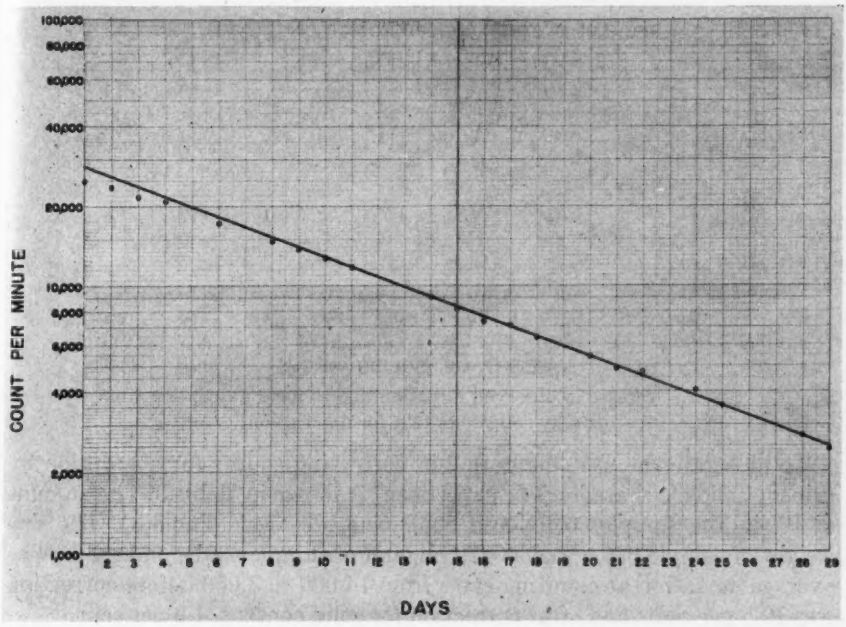

FIG. 1. *The loss in observed radiation due to coincidence counts.*

I^{131} standards, in triplicate, were prepared as described under beta counting procedure for urine. The dots are the observed values. The calculated counts/minute are represented by the line. On days 1–3, with activities greater than 20,000 counts/minute, the observed radiation is 11 per cent less than the calculated counting rate.

practice to recount, at a later date, samples which are more active than 15,000 cts./min. As pointed out in the section on gamma counting, a correction factor for loss due to coincidence counts may be established for the Geiger-Mueller tube by the usual technique of counting two sources individually, then together and computing the per cent decrease in observed counts.

Effect of urinary solids

The effect of urinary solids on the measurement of beta radiation of I^{131} is shown in Figure 2. Cups were prepared, identical in respect to final volume, amount of I^{131}, pH, NaI-carrier and gelatin; they differed in that the solid content was varied from 4 to 75 mg. The variation in urinary solids was obtained by concentration and dilution of urine before the addition of radioactive iodine. One cc. of each mixture was pipetted into weighed cups, dried and counted as described above. The absorption approximates 12 per cent in a urine of average concentration.

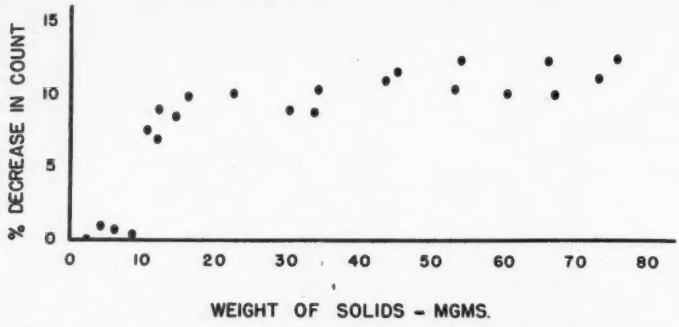

FIG. 2. Decrease in observed radiation due to mass absorption from urinary solids (*see* text for description).

To illustrate the use of Figure 2 in correcting counts for absorption by urinary solids, a characteristic experiment is shown in Table 3. The amount of I^{131} in the samples 630A and 630B was identical (Table 3). The dry weight of sample 630A was .0087 Gm. and of sample 630B, .0618 Gm. The average deviation at counting rates from 14,000 to 3,000 before correction was 10.5 per cent; and after correction for solid content, 1.9 per cent.

The effects of other chemical substances on the measurement of beta radiation

The effect of NaCl, urea, and gelatin on the measurement of beta radiation is illustrated in Figure 3. The increased loss of activity observed with these substances has not been explained; the appearance of the dried surface varied with each substance, and, in general, the surfaces in these experiments were rough and uneven. In contrast, the samples obtained with urine were smooth. It may be noted that correction for absorption as obtained with these substances would result in considerable error when applied to the experiments recorded in Table 3.

Description of the beta counting procedure for urine

Standards are prepared by diluting an aliquot of I^{131} in distilled water; the pH is adjusted to 8.0. One cc. is pipetted into each of three weighed

TABLE 3. THE EFFECT OF URINARY SOLIDS ON OBSERVED RADIATION
(*see* text for description)

Date of count	*Counts uncorrected for absorption*			*Counts corrected for absorption*		
	Sample 630A counts/min.	Sample 630B counts/min.	Per cent diff.	Sample 630A counts/min.	Sample 630B counts/min.	Per cent diff.
1-14	14040	12430	11.3	14170	14050	.8
1-15	12570	11570	8.0	12710	13080	2.8
1-16	11710	10840	7.4	11830	12240	3.3
1-17	10950	9770	10.9	11050	11030	.0
1-20	8500	7730	9.5	8590	8730	1.7
1-21	7860	7020	10.8	7940	7940	.0
1-22	7200	6370	11.6	7270	7200	1.0
1-23	6510	5950	8.6	6580	6720	2.2
1-24	5980	5260	12.0	6050	5940	1.8
1-26	5140	4480	12.7	5200	5070	2.4
1-27	4700	4030	14.2	4750	4550	4.3
1-28	4380	3780	13.7	4430	4270	3.5
1-29	3820	3480	8.9	3850	3930	1.9
1-30	3630	3270	9.8	3670	3691	.6
1-31	3250	2990	7.9	3290	3370	2.4

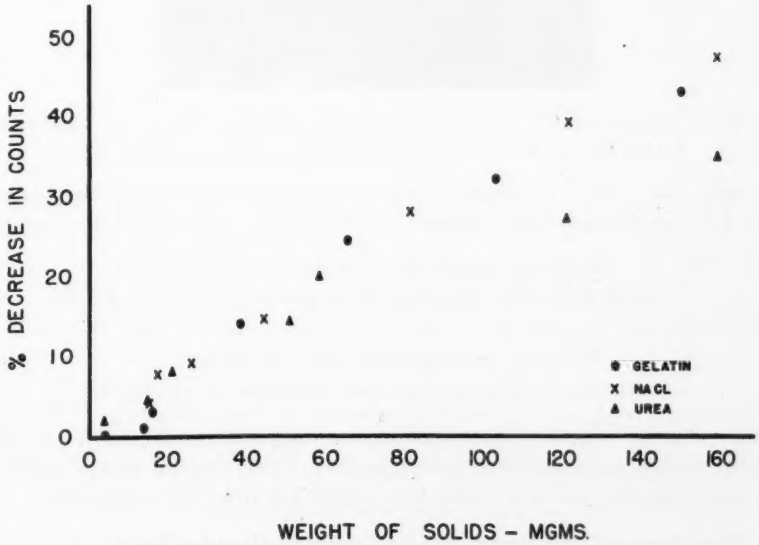

FIG. 3. The effect of sodium chloride, urea and gelatin on the measurement of beta radiation from I^{131} (*see* text for description).

cups. Approximately 0.1 mg. NaI (1 drop 0.2 per cent NaI solution) and 2–3 mg. gelatin (1 drop 10 per cent gelatin) are added to each cup. The samples are evaporated to dryness; the dried samples weighed, and determinations of the radiation made as described above. The same procedure is carried out in triplicate on 1.0 cc. samples of the patient's urine, adjusted to pH 8.0. Under these conditions the triplicate samples agree within 3

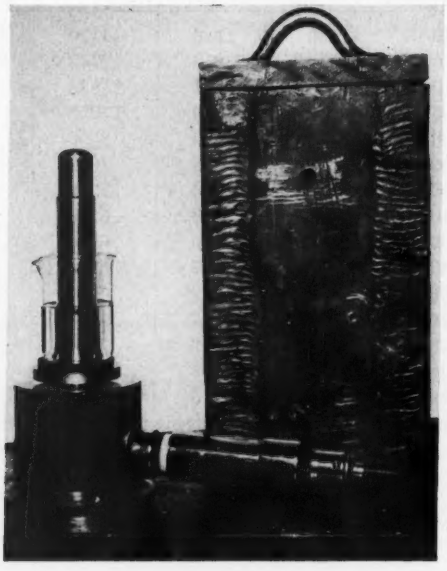

FIG. 4. The Geiger-Mueller tube, covered by a stainless steel shell, and the Marinelli beaker for measurement of urinary I^{131} by counting gamma rays.

per cent. The counts are corrected for background and for internal absorption by use of the curve shown in Figure 2. The per cent excretion

$$= \frac{\text{cc. urine volume} \times \text{corrected counts/min./cc.} \\ \text{urine} \times \text{dilution} \times \text{factor for internal absorption} \times 100}{I^{131} \text{ dose in cc.} \times \text{corrected counts/min./cc.} \\ \text{standard} \times \text{dilution} \times \text{factor for internal absorption}}.$$

Under the conditions of our technique, with sufficiently active samples, the dilution of samples to contain less than 10 mg. total solids obviates the necessity for the use of a correction factor for internal absorption.

B. Measurement of Urinary I^{131} by Counting Gamma Rays

The above described method is accurate but laborious. Furthermore because of the necessity for slow drying, results cannot be obtained before

twenty-four hours. After consultation with Mr. L. D. Marinelli and Miss Ruth Hill of the Memorial Hospital, New York, we set up the gamma counting technique employed by them (7). The Sylvania tube, GG 305, covered by a stainless steel shell and the Marinelli beaker are pictured in Figure 4.[1] Shielding is accomplished by a lead housing.

Duplicability

Fifty cc. aliquots of an I^{131} standard were pipetted into each of ten Marinelli beakers. The net counts per minute varied from 1,910 to 2,020, the mean being 1,950 (Table 4). The maximum variation between samples was 5 per cent.

TABLE 4. GAMMA COUNTS PER MINUTE OBTAINED IN 10 IDENTICALLY PREPARED SAMPLES

Beaker No.	Counts/min.	Background	Corrected counts/min.
1	1990	70	1920
2	2040	70	1970
3	1990	70	1920
4	2040	70	1970
5	1980	70	1910
6	2000	70	1930
7	2090	70	2020
8	2010	70	1940
9	2050	70	1980
10	2010	70	1940

Effect of volume on observed radiation

Figure 5 shows the variation obtained when net counts/min. are plotted against the total volume in cc. Two cc. of an I^{131} standard was pipetted into a Marinelli type beaker. Eight cc. of water was added and 4,096 counts made. The volume was increased by increments of 10 cc. of water and 4,096 counts determined at each volume change. With final volumes of 20 to 100 cc., there was a maximum variation of 4 per cent in counts per minute.

The effect of adding 10.0 cc. increments of an I^{131} standard is illustrated in Figure 6. There was a loss in observed radiation in volumes under 30 cc. and above 80 cc. For 10 cc. the loss, when compared with 50 cc., was 7.5 per cent. For 100 cc., when compared with 50 cc., the loss was 12 per cent.

[1] Made by Tracerlab, Inc. Boston, Mass. We wish to thank Dr. F. Henriques for his cooperation and help.

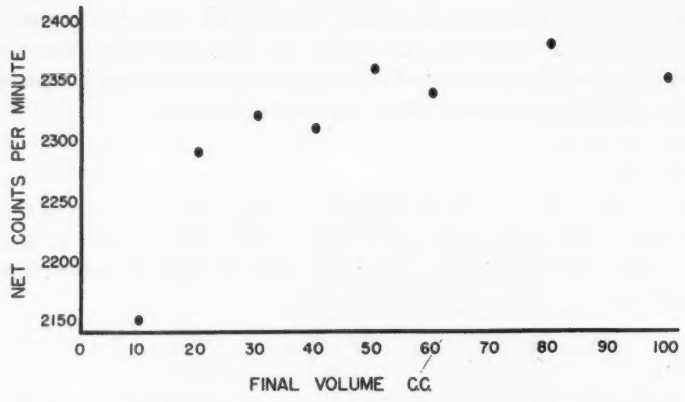

FIG. 5. The effect of volume on observed gamma radiation (*see* test for description).

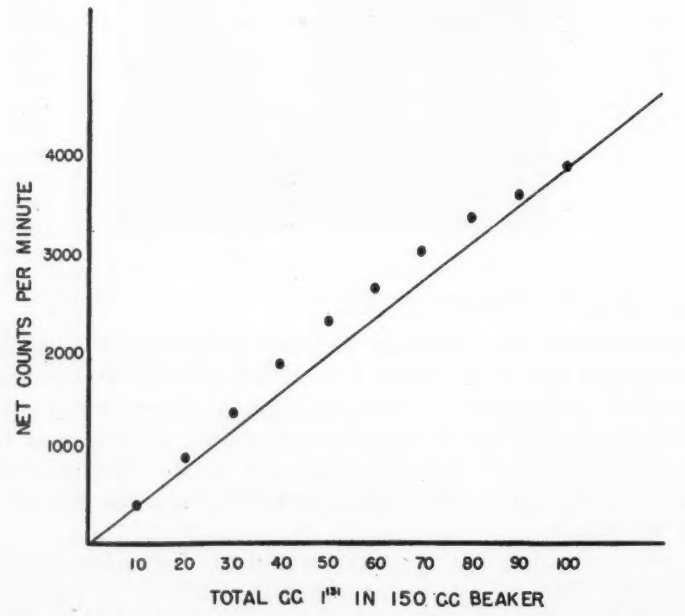

FIG. 6. The effect of adding 10 cc. increments of an I^{131} standard on observed radiation (*see* text for description).

These experiments indicate the desirability for comparing equal volumes of the unknown and standard.

Coincidence error

The recovery time of the gamma tube was calculated according to the equation

(8) (9):
$$RT = \frac{2(N1+N2-N12)}{(N1+N2)\ (N12)}$$

N1 and N2 equal net counting rates per second of similar sources counted individually; N12 equals these two sources counted simultaneously. N1 was counted first, then N2 was placed and the counts from N12 determined. N1 was removed and N2 counted. In this tube, the recovery time was 840–1,300 microseconds (Table 5). The correction factor for loss due to coincidence or per cent loss at varying counting rates is shown in Table 6.

TABLE 5. RESOLVING TIME OF GAMMA TUBE

Expt.	Source N1 (counts per minute)	Source N2 (counts per minute)	Source N12 (counts per minute)	Resolving time, micro seconds
1	3820	3870	7300	840
2	4110	3950	7410	1300

TABLE 6. COINCIDENCE ERROR

Expt.	Observed counting rate/min.	Calculated counting rate/min.	Factor
1	3610	3650	1.01
2	4980	4970	1.00
3	7410	8060	1.09
4	9910	11070	1.11
5	13600	15000	1.11
6	15500	17600	1.14

C. Comparison of Beta and Gamma Method to Determine Urinary excretion of I^{131}

In 18 subjects, the urinary excretion after an oral dose of I^{131} was determined by both the beta and gamma counting procedures described above. The per cent excretion in twenty-four hours varied from 0.41 to 64.6 (Table 7). The average difference between the two methods was 4.72 per cent with a range of 0 to 17.1 per cent; the median value was 3.8 per cent. The standard deviation was 4.37. The standard error was 0.73 and P (10), 0.78.

COMMENT

The data presented in Table 7 and its statistical evaluation show that the gamma and beta procedures give comparable results. These methods have been in use in our laboratory for the last year and a half, (1, 11, 12) and some indications of the advantages and limitations of each method for measuring I^{131} in urine have been gained.

TABLE 7. COMPARISON OF BETA AND GAMMA METHODS FOR DETERMINATION OF URINARY EXCRETION OF I^{131}

Case	Beta method Per cent	Gamma method Per cent	Per cent difference
1	.41	.41	0
2	.42	.41	2.4
3	.48	.50	3.8
4	.71	.72	.2
5	.76	.81	6.2
6	1.3	1.23	5.3
7	4.1	3.4	17.1
8	4.9	5.7	14.1
9	8.7	9.1	4.3
10	9.7	9.1	6.1
11	10.3	10.8	4.5
12	11.6	11.1	4.2
13	12.6	13.1	3.7
14	15.5	15.3	1.2
15	21.9	21.2	3.2
16	22.9	23.7	3.4
17	33.0	34.8	5.1
18	64.6	64.8	.3

The beta method is more sensitive, giving counts per microcurie approximately 250 times those obtained by the gamma method. In man, following the oral administration of 150 μc., the amount of I^{131} in various tissues and blood is minute, approximating 0.005–0.002 μc. per cc. of total serum (13). The gamma technique we have described cannot measure accurately less than 0.25 μc. Thus, when amounts of radiation are small or must be determined in blood (13) or tissue (12), the beta method is the method of choice.

In determination of urinary excretion when larger amounts of I^{131} are present, the gamma counting method is simple and results can be obtained promptly. Mass absorption and pH within the range usually observed in urines do not have to be considered. The use of the beta method for urines with activities larger than 1 microcurie per twenty-four hour excretion is cumbersome. The pH must be adjusted to the alkaline side, and mass

absorption may be a considerable factor. It is important to emphasize that in the dried sample the possibility of loss from handling exists. We, therefore, prepared all samples for the beta procedure in triplicate. The necessity of weighing cups, as well as the other factors mentioned above, militate against the usefulness of the beta procedure for the routine determination of urinary excretion.

SUMMARY

1. Two procedures used for determination of urine I^{131} radiation are described. The importance of mass absorption as a possible source of error and its correction is pointed out in the measurement of I^{131} activity by counting beta rays.

2. A study of the urinary excretion of I^{131} by the described beta and gamma counting procedures yielded agreement within five per cent.

3. The advantages and limitations of the two procedures are briefly discussed and the indications for the use of each are given.

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URINARY PREGNANEDIOL DETERMINATION AS A TEST OF PREGNANCY*

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IN 1936 Venning and Browne (1) described the isolation of a water-soluble pregnanediol complex from human pregnancy urine. In 1937 Venning, Henry and Browne (2) described the measurement of this complex and the same year Venning (3) published a gravimetric method for determination of sodium pregnanediol glucuronidate in urine, and presented evidence of its metabolism from progesterone. Venning and Browne (4) and Venning, Henry and Browne (5), using this procedure, reported the presence of significant amounts of the complex in urine from women in the luteal phase of the menstrual cycle. Subsequently, these workers and many others using the same procedure, investigated the urinary excretion of sodium pregnanediol glucuronidate in pregnant and nonpregnant women (6–18). Urinary sodium pregnanediol glucuronidate levels were reported to be of value in the diagnosis of pregnancy, of threatened abortion, and determination of the date of ovulation, all states reflecting a change in luteal activity. Since Venning's method has been reported inaccurate for small amounts of sodium pregnanediol glucuronidate (19), its application to subjects during the menstrual cycle is of doubtful value; moreover, the estimation then requires the collection of a 24-hour and preferably a 48-hour specimen of urine, with increasing opportunity for hydrolysis of the water-soluble complex.

In 1944 Guterman (20) published a qualitative colorimetric method for determination of free pregnanediol, based on the methods of Astwood and Jones (21) and of Talbot *et al.* (22). The procedure was extended and modified in subsequent papers by the same author (23, 24, 25), and developed on a quantitative basis, applicable as a diagnostic aid for pregnancy and threatened abortion.

The Guterman procedure has been widely investigated and both the analytical technique and the diagnostic value have been criticized. McCormack (26), using a slightly modified technique and applying the results qualitatively to a group of 304 patients, found the accuracy of diagnosis by the Guterman test comparable to that of the Friedman modification of the Aschheim-Zondek test. Morrow and Buena (27), following the Guter-

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man procedure, also with slight modifications and using an artificial color standard, found the test of doubtful value owning to the large number of false positives in women in the luteal phase of the menstrual cycle. Reinhart and Barnes (28) applied the Guterman test to 130 patients and reported a positive and negative error of 25 per cent. They suggested that this might be due to individual variation in progesterone metabolism. The results of the two latter groups have been criticized by Guterman (25) because Morrow and Buena used an artificial color standard of potassium dichromate and a wave-length of 530–570 mμ, whereas Reinhart and Barnes employed an artificial color standard of potassium dichromate, not comparable in intensity with that given by 0.4 mg. of pregnanediol. Huber (29) found Guterman's method highly satisfactory for extraction and for removal of acidic and phenolic compounds but preferred a chromatographic absorption to the precipitation from acetone/aqueous sodium hydroxide solution, and reported that gravimetric determination of the purified pregnanediol was superior to a colorimetric procedure. Sommerville, Marrian and Kellar (30) suggested that the Guterman method was liable to give erratic results at low levels of pregnanediol excretion and fictitiously high results in the presence of abnormally large amounts of cholesterol or 17-ketosteroids. These authors offered an alternative method of purifying and isolating the pregnanediol; the procedure for development of color was also modified, being carried out at 25° C for twenty minutes only. Bender (31) investigated pregnanediol excretion of 100 cases, diagnosed clinically as threatened abortion, and concluded that the abortion rate could be reduced by about 25 per cent if progesterone therapy was given to those showing a negative or weakly-positive Guterman test. Merivale (32) reported only a 57 per cent accuracy for diagnosis of pregnancy by the Guterman test.

The object of the present study was to evaluate the determination of urinary pregnanediol excretion as a diagnostic test for pregnancy. Since an appreciable amount of pregnanediol is excreted by women in the luteal phase of the menstrual cycle, it was decided first to investigate the excretion of this substance by a group of nonpregnant females in both phases of the cycle. The two points of interest in such levels were:—

1. Is there a statistically significant difference in pregnanediol excretion in the follicular and luteal phase of the menstrual cycle?
2. Is the excretion in early pregnancy sufficiently different from that of nonpregnant females, in the luteal phase of the menstrual cycle, to enable the determination to be used as a diagnosis of pregnancy?

PROCEDURE FOR THE ESTIMATION OF PREGNANEDIOL

The quantitative procedure of Guterman (25) was used as a basis, but the following modifications were found to be advantageous:—

A. *Hydrolysis and extraction.* In accordance with the suggestion of Sommerville *et al.* (30), 10 ml. of concentrated hydrochloric acid was added to the urine/toluene mixture at the point of boiling, to prevent unnecessary exposure of the pregnanediol to strong acid. To provide more thorough separation of pregnanediol three toluene extractions were carried out in place of only one.

B. *Precipitation of impurities.* In the Guterman procedure toluene/water emulsions caused some difficulty but could be avoided by prolonging the time required for separation and by drying any residual emulsion with anhydrous sodium sulphate, which was then removed by filtration. The sodium sulphate residue was washed twice, on the filter paper, with small amounts of toluene.

C. *Precipitation of pregnanediol.* Whereas Guterman allowed the acetone/sodium hydroxide solution containing pregnanediol to stand for one hour at 5° C, it was found advantageous to lengthen the time to sixteen hours at a temperature of 10° C.

D. *Isolation of pregnanediol.* The precipitated pregnanediol, on the fritted glass filter, was washed with 10 ml. of petroleum ether to remove 17-ketosteroids, as suggested by Davis and Fugo (33).

E. *Development and measurement of color.* The pregnanediol, finally separated, was dissolved in 5 ml. of pure ethanol. Convenient-sized aliquots (0.2–1.0 ml.) of the solution were pipetted into 25 ml. test tubes, the ethanol evaporated in boiling water, the tubes allowed to cool, and 10 ml. of concentrated sulphuric acid buretted into each. In contrast with the experience of Sommerville *et al.* (30), it was found desirable to allow forty-five minutes for development of maximum color. The color value was found to be constant during a 45–60 minute period after addition of the acid. Color intensity was read in a Coleman No. 11 Universal spectrophotometer, at a wave length of 430 mμ, against a blank of concentrated sulphuric acid. Calibration curves were prepared daily, using a standard solution of sodium pregnanediol glucuronidate in 90 per cent ethanol. Experiments in which pregnanediol was separated from the sodium glucuronidate indicated that the color developed was due entirely to the pregnanediol. The conversion figure for expressing sodium pregnanediol glucuronidate in terms of pregnanediol was derived directly from the molecular weights of the two compounds and was 0.597.

To avoid possible contamination from rubber or cork stoppers, standard taper glassware was used for all hydrolysis and extraction procedures.

All reagents were tested for possible contribution to color formation and were found to be without effect. Six specimens of male urine, subjected to the complete procedure, gave zero readings. The method was checked by recovery experiments, with the results shown in Table 1.

The results of the above recovery experiments tend to substantiate the statement of Sommerville *et al.* (30) that the procedure is of doubtful accuracy for small amounts of pregnanediol.

Collection of urine specimens. It is generally difficult to obtain 24-hour specimens of urine; for convenience, first morning specimens have obvious advantages. It seemed useful to determine whether the estimation of pregnanediol in such specimens was of diagnostic value. All results reported here have been obtained with first morning specimens. Urine was preserved by saturation with benzoic acid, a substance which does not in-

TABLE 1. RECOVERY OF PREGNANEDIOL

Test vehicle	Sodium pregnanediol. glucuronidate added before acid hydrolysis, expressed as mg. pregnanediol	Per cent recoveries: mean of 3 determinations
100 ml. water	2.5	96
	1.0	76
	0.5	70
100 ml. male urine	5.3	110
	2.4	91

terfere with the determination of pregnanediol. Estimations were made as soon as possible after collection.

CLINICAL MATERIAL

1. *Control group.* To arrive at a "base-line" of pregnanediol excretion by normal, nonpregnant females, a group of 44 persons (age 19 to 36 years) was studied. The estimated length of the menstrual cycle was recorded, together with the actual length of two consecutive cycles. A first morning specimen of urine was collected on the ninth, tenth or eleventh day, and the twentieth, twenty-first or twenty-second after the beginning of menstruation. A study of the recorded cycles allowed an estimation of the time of ovulation (approximately fourteen days prior to the beginning of the second menstrual period). Only those individuals whose second specimen could be assumed to be postovulatory were included in the final group which totalled 38 persons.
2. *Clinical group.* A group of 59 women, whose menstrual period had been missed for four to twelve weeks and who were attending the Pre-Natal Clinic, Toronto General Hospital, furnished first morning specimens of urine. Of this number, 52 were pregnant.

RESULTS

Urinary pregnanediol levels are summarized in Table 2. Using Fisher's "t" test, the t values for the differences between the means of the two control levels was 3.1, a figure which is highly significant. The distribution of the logarithms of pregnanediol values is shown in Figure 1.

Use of pregnanediol levels for the diagnosis of pregnancy. Although there was a marked difference between the mean values of pregnanediol concentrations in urine specimens from pregnant women and from those in the

luteal phase of the menstrual cycle, there was overlapping of individual levels. The level which is to be considered as diagnostic of pregnancy is debatable. Guterman first fixed the "positive" level as 1.0 mg. of pregnanediol per 100 ml. of urine, or 15–20 mg. total for 24-hour excretion; he later proposed 0.4 mg. per 100 ml. or 6–8 mg. per 24-hour specimen. Both levels were selected by Guterman on the basis of personal judgment, arrived at by a study of the observed values. It seemed desirable to have a

TABLE 2. URINARY PREGNANEDIOL VALUES

Group	Clinical condition	Number of subjects	Pregnanediol concentration, mg./100 ml. of urine	
			Range	Mean and standard deviation
Control: School of Hygiene Staff and T.G.H. Dietitians	Follicular phase of menstrual cycle	38	0.07–0.51	0.22 ±0.119
Control: School of Hygiene Staff and T.G.H. Dietitians	Luteal phase of menstrual cycle	38	0.06–1.81	0.42 ±0.344
Clinical: Outpatients, Pre-Natal Clinic T.G.H.	Pregnant 4–12 weeks since last menstrual period	52	0.30–9.50	2.44 ±1.864

critical level which would be independent of subjective opinion and which would be determined by statistical treatment. A critical level of this type has been calculated in the following way:—The actual pregnanediol levels in the three series reported above are not normally distributed but the logarithms of the values are approximately normally distributed. A critical level, lying between the two means, was selected so that the probability of a pregnant woman being classified as "nonpregnant" would be equal to the probability of the opposite type of error, assuming a normal distribution of logarithms of the observed values. The estimated error for either type of mistaken diagnosis, arrived at from this critical level, would be about 12 per cent. In other words, the theoretical accuracy of the test would be about 88 per cent.

Using the two critical levels proposed by Guterman and that which has been calculated from our data, all subjects have been classed as "pregnant" or "nonpregnant" depending on whether the observed value was

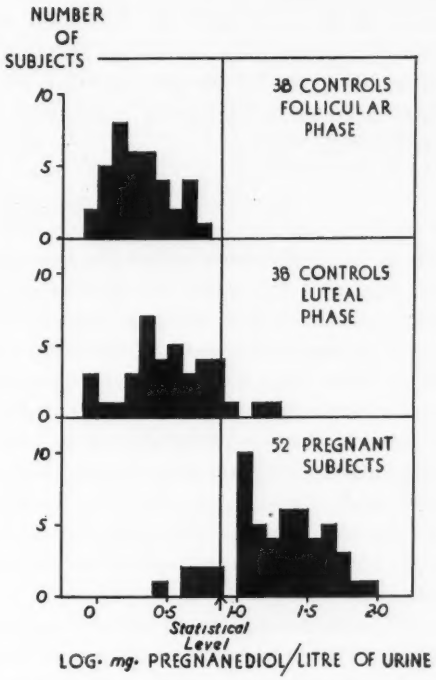

FIG. 1. Distribution of logarithms of pregnanediol levels in urine. The "statistical level" is the value which has been selected statistically, to be used for diagnosis of pregnancy.

TABLE 3. DIAGNOSTIC VALUE OF PREGNANEDIOL DETERMINATIONS

Group	Number of subjects	Correct diagnosis		
		Guterman's level 1.0 mg./100 ml.	Guterman's level 0.4 mg./100 ml.	Statistical level 0.77 mg./100 ml.
		Per cent	Per cent	Per cent
Control: follicular phase	38	100	87	100
Control: luteal phase	38	95	63	92
Clinical: early pregnancy	52	87	98	87

above or below the particular critical level. The classification thus obtained was compared with the clinical information for each subject, to determine the accuracy of diagnosis given by the pregnanediol test. The percentages of accuracy for the three series of results, calculated from each critical value, are given in Table 3.

DISCUSSION

The reliability of the diagnosis of pregnancy by the determination of pregnanediol depends primarily on whether the amount of the substance excreted during pregnancy is different from that in the nonpregnant state. The results reported here show that there is considerable overlapping between levels observed in the luteal phase of the menstrual cycle and those found in pregnancy. If there were a distinct gap, the use of the pregnanediol test would be simple. Since that is not the case, a critical level has to be selected and it is advisable to do so by a procedure which is free from subjective error. Statistical treatment of our data yielded a critical level which, theoretically, has an error of 12 per cent. Actually, by the application of this level to the data reported above, 8 per cent of nonpregnant subjects would be classified as "pregnant," and 13 per cent of pregnant women would be classified as "nonpregnant." It should be noted that evaluation of our data on this basis gave more false negatives than false positives.

Various statements of the reliability of biologic tests for pregnancy have been made. In this laboratory the accuracy of the Friedman modification of the Aschheim-Zondek test, conducted on rabbits, was found to be about 98 per cent (34). Two rabbits were used for each test and the test was repeated if the two animals gave discordant results. Guterman reported that the Friedman test was 87 per cent accurate (23). The female South African frog (Xenopus laevis) test has been said to be 99.6 per cent reliable for diagnosis (35). It is likely that the pregnanediol test cannot be considered as accurate as the established biologic procedures, but the chemical method has the advantage in not requiring the maintenance of animals. In our opinion it is a useful procedure, particularly if it is applied with the proviso recommended by Guterman, that the test be considered positive only after at least one missed menstrual period. We would add an additional proviso, that the result be evaluated in terms of a statistically obtained critical level.

The pregnanediol test might be even more reliable if 24-hour specimens were used, but, in practice, these are difficult and often impossible to obtain. A previous report from this laboratory has indicated a relation between the concentration of 17-ketosteroids in the urine of normal males and the specific gravity of the urine (36); no similar relation was found for pregnanediol.

SUMMARY AND CONCLUSIONS

A modification of the Guterman procedure has been developed and used for the determination of urinary pregnanediol. The procedure is probably not accurate for amounts less than 0.5 mg./100 ml. of urine; figures of this order have been reported here but have been used with reservation. Determinations were made on morning specimens of urine preserved with benzoic acid, from 38 nonpregnant women in both phases of the menstrual cycle, and from 52 women who were four to twelve weeks pregnant. Although there was overlapping of individual values between the two phases of the menstrual cycle and between the luteal phase and early pregnancy, the means of the three sets of values were significantly different. A statistical method for selecting a critical "positive" level is proposed; using this level, the diagnosis of pregnancy from pregnanediol concentration in the urine was theoretically 88 per cent accurate. In the cases reported there were 8 per cent false positives and 13 per cent false negatives. Although the procedure is not as reliable for diagnostic purposes as are several biologic methods, it is considered useful, particularly when animal facilities are not available.

Acknowledgments

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PSEUDOHYPOPARATHYROIDISM

REPORT OF A CASE WITH LATE MANIFESTATIONS

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IN 1942 Albright (1) and his co-workers first described a condition resembling hypoparathyroidism in which there appeared to be no deficiency of the parathyroid secretion but a failure of the body end-organs to respond to the hormone. Three patients were reported who presented certain characteristic physical features with histories of convulsions or other symptoms of parathyroid deficiency, and in whom the results of chemical studies were identical with those of hypoparathyroidism except for complete lack of response to administered parathyroid hormone. In one patient a biopsy revealed apparently normal parathyroid tissue. It was postulated that a normal amount of parathyroid hormone was secreted but that the tissue end-organs were refractory to the hormone. The name "pseudo-hypoparathyroidism" was given to this condition. It has also been called an example of the "Seabright-bantam syndrome." The Seabright bantam is a species of fowl in which the male shows female plumage and comb. Normal testes have been demonstrated, and this species has been considered the classic example of end-organ refractoriness to a normal amount of circulating hormone. Review of Morgan's original papers (2, 3) on the Seabright bantam leaves some doubt as to whether this bird represents a true example of end-organ refractoriness. It was conclusively shown that following castration the male bantam developed male plumage and comb. This suggests an endocrine-inhibiting mechanism rather than true end-organ refractoriness. Perhaps the term "Seabright-bantam syndrome" should be dropped but, nevertheless, the concept of a refractory end-organ producing a picture resembling a glandular deficiency is valid. A simpler example is the beardless American Indian who has a normal amount of testosterone.

Four additional cases of pseudohypoparathyroidism have been reported; one by Sprague (4) and his co-workers; two by Selye (5); and one by Peterman and Garvey (6).

Because of the rarity of the condition and because of the important

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theoretical implications of a condition of end-organ defect, such cases deserve further study. The following case is presented as an example of pseudohypoparathyroidism diagnosed late in life, and showing some of the late manifestations of the disease:

CASE REPORT

V. S. (MCV #37753), a 49-year-old white male, was admitted to the Medical College of Virginia Hospital on April 8, 1948, complaining of pains in the extremities.

Family history revealed that one brother died of diabetes and one of tuberculosis. There was no other history of endocrine disorders, and the patient's parents had been normal as far as he knew.

The patient was born at term. No other details of the pregnancy were known. He was always a "fat" baby, having weighed 45 pounds at the age of nine months (Fig. 1). He

FIG. 1. The patient at age nine months, weight 45 pounds. Note that the fat, rounded head and short stubby extremities are apparent at even this early age.

had "never grown as other children," always being short and fat, with a round face and short limbs. Between the ages of 17 and 20 he had convulsive seizures which varied from three to four daily to once a month. He was never incontinent with these seizures. No cause was found, and he "outgrew" them and had no recurrence, with the possible exception of one fainting spell at the age of 37. At the age of 25 he first noticed painless firm, warty nodules on the fingers which gradually increased in size and number. At the age of 28 he noticed pain and stiffness in the right elbow. This was intermittent, but during the following years he developed increasing stiffness and shooting pains in both shoulders, elbows, neck, back, hips, and feet. These had finally incapacitated him a few months before his admission.

The patient had worn glasses for forty years, but during recent months had noticed progressive blurring and dimness of vision. He had had mild periodic asthma for about

forty years. One year before admission he had first noticed a tremor of the hands and a shuffling type of gait. The patient had had a normal sex life, was married and the father of two apparently normal children. He had never received any endocrine treatment.

Physical examination revealed a short, stocky male, whose gait was slightly propulsive, with the elbows held in slight flexion. Body measurements were as follows: weight 145 lbs., height 59½ in., span 57 in., from symphysis to vertex 31 in., from symphysis to floor 28½ in., circumference of the head 23 in., circumference of the chest 37 ¾

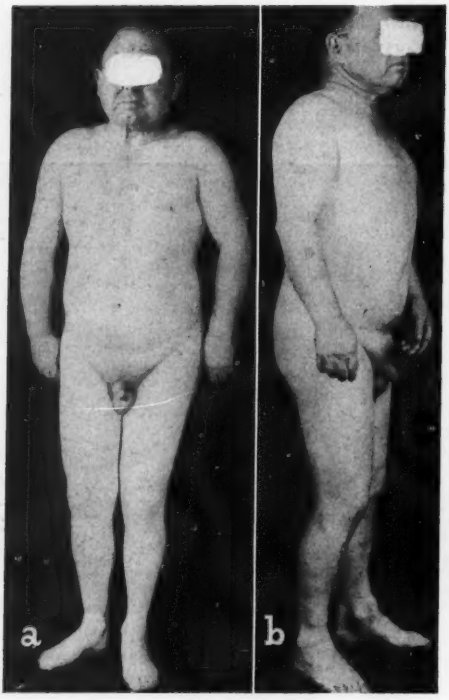

FIG. 2 (a and b). The patient at the time of admission, showing the characteristic physical appearance as described in the text.

in., and circumference of the abdomen 36 in. The blood pressure was 112/75. Temperature was 98.6°, pulse 72, and respiration 18. The face was round, the neck short and thick, and the limbs appeared short in relation to the trunk (Figs. 2a and 2b). Hair distribution and skin texture were normal. The pupils were round, equal, and reacted normally. Bilateral early lens opacities were present beneath the anterior and posterior capsule. The fundi were normal. The ears, nose, and throat were not remarkable. Dental examination revealed all the teeth to be small, especially the bicuspids. There was a marked overlapping and all the teeth showed advanced abrasion. The left lower second bicuspid and the upper right cuspid were unerupted, although roentgenograms showed no evidence

of significant impaction. These teeth normally erupt between the ages of 10 and 13 years. The lungs showed moderate emphysema and scattered expiratory wheezes. The heart was normal and the abdomen not remarkable. The genitalia were small but normally developed.

Over the first three fingers of both hands, over the dorsum of both feet, and attached to the Achilles tendons there were numerous stony hard subcutaneous nodular plaques. These were non-tender and immobile, being apparently firmly attached to the deeper structures (Fig. 3). There was limitation of motion of the shoulders, spine, hips, and feet.

FIG. 3. Palmar view of the left hand showing the subcutaneous calcifications.

Neurologic examination was not remarkable except for a gross "pill-rolling" type of tremor of the forearms and hands, more marked on the right, present at rest, but disappearing with intentional movements. Chvostek and Trousseau signs were absent.

Laboratory studies showed normal blood counts and urinalyses. The blood chemistry was as follows: serum calcium 6.8 mg. per cent, serum inorganic phosphorus 4.5 mg. per cent, total serum protein 7.7 Gm. per cent with albumin 4.3 Gm. and globulin 3.4 Gm., serum cholesterol 196 mg. per cent, alkaline phosphatase 1.8 Bodansky units, and acid phosphatase 2.7 King-Armstrong units. The erythrocyte sedimentation rate was 24 mm. in one hour and the Wassermann test was negative. The urinary Sulkowitch test (7) was negative. The 17-ketosteroid excretion was 9.3 mg. in twenty-four hours. The phenolsulphonthalein excretion was 62 per cent in two hours. The basal metabolic rate was minus 11 per cent. The electrocardiogram was not remarkable except for a P-R interval of 0.22 seconds, and low T waves in leads 1, CR_4 and CR_5.

Roentgenograms of the skull revealed a normal cranial vault with a normal sella turcica and pineal body but extensive bilateral calcification in the parietal and occipital

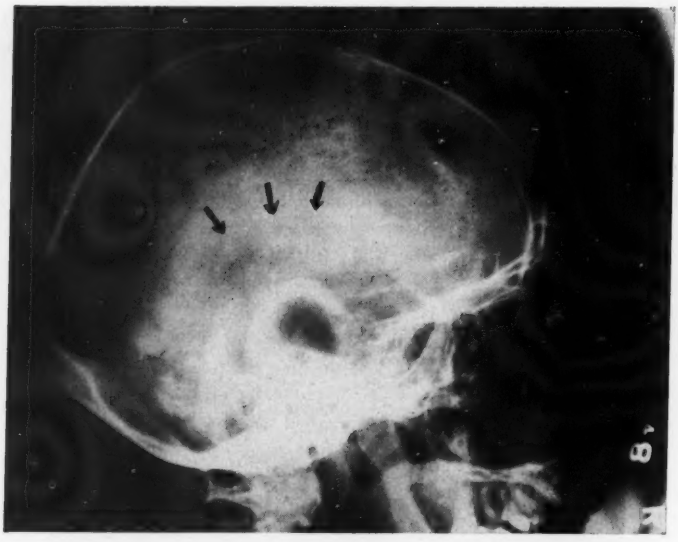

FIG. 4. Roentgenogram of the skull showing calcifications in the region of the basal ganglia.

FIG. 5. Roentgenogram of the right leg showing extensive soft tissue calcinosis.

regions (Fig. 4). Roentgenograms of the hand, legs, and feet showed extensive calcium deposits in the soft tissues, tendons, and bursae (Fig. 5). An intravenous pyleogram showed normally functioning kidneys without calculi. There was some hypertrophic lipping of the lumbar vertebral bodies.

Two of the subcutaneous plaques on the left hand were removed for study. On dissection they had the consistency of bone. Biochemical assay showed the material to consist chiefly of calcium phosphates of about the same composition as bone. Histologic study revealed bony trabeculae showing osteoblastic activity, with areas of abundant fibrous tissue and scattered cells resembling hematopoietic elements (Figs. 6a and 6b).

An Ellsworth-Howard test (8) was carried out, 2.0 cc. (200 units. U.S.P.) of the parathyroid hormone being given intravenously. The results are shown in Table 1 and are compared with that of a normal control. No phosphorus diuresis occurred in our patient whereas there was a definite phosphorus diuresis in the control with a transitory fall in

TABLE 1

Time	Urinary phosphorus		Urine calcium		Serum inorganic phosphorus		Serum calcium	
	Patient V.S.	Control	Patient V.S.	Control	Patient V.S.	Control	Patient V.S.	Control
	mg./24 hrs.	mg./24 hrs.	mg./24 hrs.	mg./24 hrs.	mg. %	mg. %	mg. %	mg. %
8- 9	50.5	14.0	3.7	1.4				
9-10	50.5	24.2	3.9	10.3	1.5	4.1	8.0	10.6
10 a.m.	200 units Parathyroid extract I.V.							
10-11	36	61.5	3.6	2.4	1.6	3.8	8.1	10.9
11-12	29	22.6	2.4	1.0	1.5	4.1	8.4	11.8
12-1	21.7	12.6	<2.0	Trace				
1-2	13.8	39	<2.0	9.0	2.0	4.2	8.2	13.8

the serum inorganic phosphorus. The serum calcium of the patient remained essentially unchanged, whereas that of the control showed a late rise. In our opinion, these results signify that the patient was refractory to the parathyroid hormone.

It was thought that the patient presented the findings of pseudohypoparathyroidism, which had been present since birth, with cataracts and extensive tissue calcification as the late results of a life-long disturbance of calcium and phosphorus metabolism.

The administration of AT-10 (dihydrotachysterol) was begun on April 17, 1948, and the patient was discharged on May 10, 1948, to be followed in the Out-Patient Department. Graphic tabulation of serial calcium and phosphorus determinations during this and subsequent therapy is presented in Figure 7. At first the large dose of 3.0 cc. (3.75 mg.) of AT-10 daily was given. As the serum calcium rose, this was reduced to 1.5 cc. (1.87 mg.) daily, and later to 0.5 cc. (0.625 mg.) daily after calcium chloride, 4.0 Gm. daily, was added. On the latter maintenance schedule the serum calcium was maintained at an approximately normal level. Serum phosphorus had not been abnormally high before treatment but showed some lowering while AT-10 was being given.

FIG. 6. Photomicrographs of the subcutaneous plaque removed from the left hand. Note the bony trabeculae, with osteoblastic activity at the periphery.
a) ×90. b) ×400.

Because of the expense of such dosage of AT-10 and because Sprague (4) and others have shown that vitamin D is as effective as AT-10 in pseudohypoparathyroidism, it was decided to try vitamin D in large doses instead of AT-10. On June 30, 1948, AT-10 was discontinued and vitamin D, 100,000 units daily, was begun. The same dosage of calcium chloride was continued. On this program there was a further rise in the serum calcium with a fall in serum inorganic phosphorus. Because of the marked rise in the serum calcium, the vitamin D was reduced to 50,000 units daily and finally to 50,000 units every other day. The daily dose of 4.0 Gm. of calcium chloride was continued. The comparison of these two therapeutic agents will be further discussed below. On this schedule the serum calcium returned to approximately normal levels.

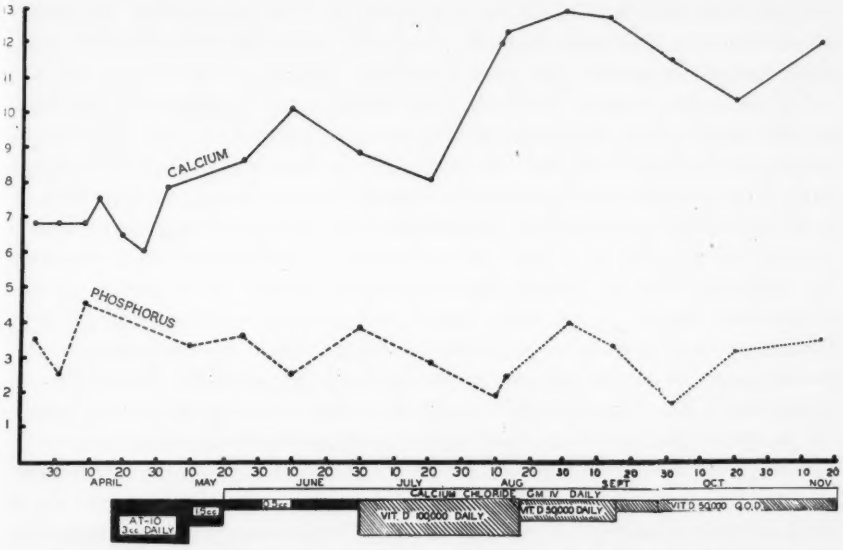

FIG. 7. The effect of dihydrotachysterol (AT-10) and vitamin D on the serum calcium and phosphorus in patient V. S.

For some reason the patient's pains in the back and limbs improved considerably after he began treatment. Presumably his pain was caused by irritation of soft tissues and nerve fibers by the extensive deposits of calcium present. No change in the calcium deposits already present was expected, and none was observed. Nevertheless, the patient considered himself very much improved and was pleased with his treatment. He returned to work as storekeeper and has not missed any time from his job for the past six months.

DISCUSSION

Pseudohypoparathyroidism presents most of the clinical features seen in true hypoparathyroidism (9–19). Tetanic manifestations or convulsions associated with the chronic hypocalcemia are the most outstanding symptoms in both conditions. The tendency to cataract formation and to the

development of intracerebral calcification is common to both conditions. The blood in both shows a lowered serum calcium with elevated serum phosphorus and a normal phosphatase level.

Certain definite criteria differentiate the two conditions. All of the reported cases of pseudohypoparathyroidism have shown a characteristic physical appearance (1, 4, 5). The stature is short, with particular shortening of the long bones in relation to the trunk. The face is round with the neck thick and tapering. The hands are stubby and fat with short rounded fingers. The metacarpal bones may be particularly short.

While intracerebral calcifications occur in both conditions, extensive calcification in the subcutaneous tissues of the body is said to be very characteristic of pseudohypoparathyroidism (20).

The final diagnosis of pseudohypoparathyroidism depends on the demonstration of refractoriness to the intravenous administration of parathyroid hormone. Ellsworth and Howard (8) have shown that both the normal individual and the true hypoparathyroid patient respond to a test dose of parathyroid hormone with an immediate and striking phosphorus diuresis followed by a fall in serum phosphorus and later by a rise in serum calcium. The patient with pseudohypoparathyroidism shows no response at all, being refractory to the parathyroid hormone. When this test is performed, previous recent parathyroid hormone therapy must be excluded, as the development of antihormones can produce a negative result. Renal disease which might interfere with phosphorus excretion must likewise be excluded.

The pathogenesis of pseudohypoparathyroidism is entirely unknown. Most of the previously reported cases have been in very young individuals and in our patient the process would seem to have been present since birth. The skeletal alterations suggest that this is a congenital disturbance. The extensive degree of tissue calcification may possibly reflect the lifelong duration of the process as compared with the briefer duration of true hypoparathyroidism, a disease acquired later in life.

It is of interest that our patient had convulsive seizures for several years in earlier life but overcame these spontaneously. At no time during our period of observance were the Chvostek and Trousseau signs positive, in spite of very low serum calcium levels. It is suggested that some compensatory adjustment in the nervous system may have taken place, alleviating the excessive irritability usually accompaying hypocalcemia. Likewise, the serum phosphorus in our patient was within normal limits before treatment. Albright (21) postulated that cataract formation and tissue calcification result more from hyperphosphatemia than from hypocalcemia. It is possible that our patient had previously had higher serum phosphorus values which had been reduced by some homeostatic mechanism.

Our patient showed definite evidence of early Parkinsonism. There was

no history of any illness resembling encephalitis. Disturbances of cerebral function have been commonly observed in true hypoparathyroidism (13, 22) and their relationship to intracerebral calcification has been discussed (13, 23, 24). The consensus seems to be that the disturbed cerebral function and the calcification both result from brain damage due to metabolic causes. It seems likely that such damage had occurred in the basal ganglia of our patient. No change in the signs of Parkinsonism was observed after treatment.

Histologic study of the calcific fragment removed from the finger of our patient showed a structure remarkably similar to true bone, and what appeared to be true osteoblastic activity. The mechanism of the formation of heterotopic bone is not clear. Duncan (25) states that it may occur in calcinosis universalis. Rothstein and Welt (26) cite a case in which a large sequestrum histologically consisting of bone lamellae was removed from calcinosis of the anterior abdominal wall. The question arises as to whether fibroblasts of the subcutaneous tissues have taken over osteoblastic function in the presence of altered calcium and phosphorus metabolism.

As to the choice of preparations for treatment, consideration must be given to the mechanism of their action and to the aims of treatment. When tetanic or convulsive manifestations are present, elevation of the serum calcium is obviously the most urgent need. In true hypoparathyroidism this may be accomplished either by AT-10 (11, 14, 16) or large doses of vitamin D (17), together with supplementary oral calcium. If hyperphosphatemia is important in the causation of cataracts and tissue calcification, as Albright believes (21), AT-10 would appear to be preferable because of its greater effect in promoting phosphorus excretion and directly lowering the serum phosphorus (11). Usually a small maintenance dose of AT-10 is sufficient. Where larger doses are necessary, the expense of such treatment may be considerable. In these cases, the simultaneous use of vitamin D with a smaller dose of AT-10 seems reasonable.

In pseudohypoparathyroidism AT-10 is effective, but larger doses are required (1). This is probably due to the fact that these patients are as refractory to the phosphorus-excreting action of AT-10, as they are to the parathyroid hormone. AT-10 is effective then only in so far as it increases calcium absorption. This effect is essentially the same as that of vitamin D. Sprague (4) and his co-workers have shown that in pseudohypoparathyroidism the effects of AT-10 and vitamin D are identical. From our observations with the patient reported here, it appears that vitamin D is as effective as AT-10 and somewhat less expensive. Vitamin D in large doses, combined with supplementary oral calcium, would appear to be the treatment of choice.

SUMMARY

1. A case of pseudohypoparathyroidism, first diagnosed at the age of 49, is reported.

2. The clinical features of pseudohypoparathyroidism are presented.

3. Very extensive tissue calcification, spontaneous cessation of convulsions, and a serum phosphorus level within normal limits were unusual features in this case. Their possible relationship to the long duration of the disease is discussed.

4. Large doses of vitamin D, with supplementary oral calcium administration, appeared to be a suitable form of treatment.

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CELLULAR INVOLUTION IN THE THYROID GLAND*

SIGNIFICANCE OF HÜRTHLE CELLS IN MYXEDEMA, EXHAUSTION ATROPHY, HASHIMOTO'S DISEASE AND THE REACTIONS TO IRRADIATION, THIOURACIL THERAPY AND SUBTOTAL RESECTION

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ALTHOUGH the eosinophilic cells present in the so-called Hürthle cell tumors probably bear little relation to the interfollicular cells in the thyroids of young dogs described by Hürthle many years ago, prolonged usage has inextricably associated his name with these elements. It has been pointed out that Hürthle cells represent a peculiar type of cellular transformation which occurs in a variety of conditions, but their existence in certain tumors (1) has been much more widely recognized than their presence in non-neoplastic lesions of the thyroid.

A review of the numerous specimens of thyroid received at the Army Institute of Pathology between August 1942 and January 1948 made it clear that the development of Hürthle cells takes place not only in thyroid hyperplasia, in Hashimoto's disease and in exhaustion atrophy but as a consequence of irradiation, administration of thiouracil and partial thyroidectomy. This study was designed to determine the common factors which, operating under apparently widely diverse circumstances, result in the same phenomenon. It is to be hoped that investigation of this peculiar cellular reaction may aid in the formulation of certain biologic concepts which will apply to the other endocrine glands as well as to the thyroid.

Certain epithelial alterations, the most prominent of which is the formation of Hürthle cells, will be referred to under the designation "cellular involution." Cellular involution should be distinguished from follicular involution, that process in which colloid is redeposited while follicular epithelium is reconverted from the active to the resting state.

The classic Hürthle cell is eosinophilic, but variants with a cytoplasm which is foamy or finely stippled with granules are also encountered. Hürthle cells tend to be square rather than columnar and often have a

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distinct cellular border. Their being closely packed, their size and their shape give a "solid" appearance to areas in which they are abundant. Bizarre, large and distorted nuclei are common; often the nuclei are pyknotic and crenated, with irregular scalloped indentations where the cytoplasmic mass impinges on the nuclear membrane. Follicular conformation is often preserved, but the follicles tend to be small and round and may contain bright eosinophilic colloid material, often of a shade identical with that of the cellular cytoplasm. In advanced stages of involution only scattered nests and cords of polyhedral eosinophilic cells remain in the stromal connective tissue.

In some of the thyroids from patients with myxedema (Plate 1; Fig. 1) no normal parenchymal tissue was left. In the fibrotic glands there were occasional nests of Hürthle cells which lacked a follicular pattern and were accompanied by lymphocytic infiltration. The picture in so-called exhaustion atrophy was comparable, but involution was neither as uniform nor as profound (Plate 1; Fig. 2). It should be pointed out that the time honored term "exhaustion atrophy" indicates that the involutionary nature of the process has been recognized.

Although the reaction of the thyroid to various types of irradiation has been subjected to close study, no typical or characteristic histologic pattern has emerged. In some of the specimens which were studied after irradiation, features of atypical nodular adenomatous goiter were recognizable in addition to many foci of Hürthle cells (Plate 1; Fig. 3 and 4) and other involutionary elements.

Nodules of Hürthle cells were not infrequently seen in diffuse toxic goiter, especially when the hyperthyroidism was of long standing. It was sometimes possible to recognize in hyperplastic thyroid epithelium the early stages of transformation to Hürthle cells, particularly when the alteration was focal. The tall columnar cells broadened, the cytoplasm became clear or eosinophilic and the nuclei exhibited deviations from the normal.

The involutionary changes consequent to the administration of thiouracil are to be compared with those in simple hyperplasia for two reasons: first, because the drug is used for the treatment of toxic hyperplasia, and second, because it itself provokes hyperplasia of thyroid epithelium. In the glands of a few patients treated with thiouracil there was extensive formation of Hürthle cells (Plate 2; Fig. 5) in addition to the peculiar intensification of pre-existing hyperplasia which is often associated with the administration of this drug. The entire thyroid of one patient with hyperthyroidism who was treated with thiouracil consisted of nodules of Hürthle cells (Plate 2; Fig. 6) arranged in papillary conformations so bizarre that a diagnosis of neoplasia had to be considered.

The tissue left after subtotal resection for hyperthyroidism exhibited transformation to Hürthle cells in several instances. In some cases the residual tissue assumed the form of a pleomorphic nodular goiter (Plate 2; Fig. 7) in which nodules of Hürthle cells (Plate 2; Fig. 8) were conspicuous. In others (Plate 3; Fig. 11) the transformation was so uniform that the over-all pattern was indistinguishable from that of Hashimoto's disease. In a few instances thyroid parenchyma, composed almost entirely of Hürthle cells, had proliferated so extensively after repeated operations for recurrent exophthalmic goiter that the appearance of a tumor was simulated.

In several cases more than one factor could have been held responsible for the involutionary changes. For example, Figure 9 (Plate 3) shows a focal aggregation of Hürthle cells in the thyroid of a patient who had undergone resection twice and had received a course of thiouracil. This nodule could have resulted from prolonged toxic hyperplasia, from the treatment with thiouracil or from the operations.

The thyroid in Figures 10 and 11 (Plate 3) is from a patient who had had several operations and a course of roentgen therapy. Tissue which had been removed from the thyroid of this patient eight and four years prior to the final operation was available for study. Early involutionary changes were recognized in the earliest specimen, and there was unequivocal formation of Hürthle cells in the tissue removed four years later (Fig. 10). Eight years after the original operation, when the third resection was carried out, examination showed extreme involution and the formation of so many Hürthle cells that the resultant picture (Fig. 11) duplicated exactly the pattern seen in Hashimoto's disease (Fig. 12).

Lymphocytic infiltration was a frequent concomitant of cellular involution. In the study of Hashimoto's disease so much attention has been paid to the lymphoid component that the far more significant epithelial alterations have received due emphasis only relatively recently (2). Transformation to Hürthle cells and desquamation into the follicular lumens was more marked in Hashimoto's disease than in most other types of in-

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FIG. 1. (Neg. No. 103291; Acc. No. 96365) Myxedema. Most of the thyroid parenchyma has been replaced by connective tissue. A few nodules of Hürthle cells remain. (×185)

FIG. 2. (Neg. No. 103281) Exhaustion atrophy. Thyroid of an elderly woman who died of carcinoma of the breast. Dark Hürthle cells form solid nests. Cells exhibiting an intermediate stage of involution are present in the few remaining follicles. (×185)

FIG. 3. (Neg. No. 103278; Acc. No. 42121) Reaction to irradiation. Numerous Hürthle cells are present in hyperplastic epithelium. (×185)

FIG. 4. (Neg. No. 103280) Reaction to irradiation. Darkly eosinophilic Hürthle cells are abundant in a gland exhibiting many pleomorphic nodules composed of atypical and bizarre elements. (×185)

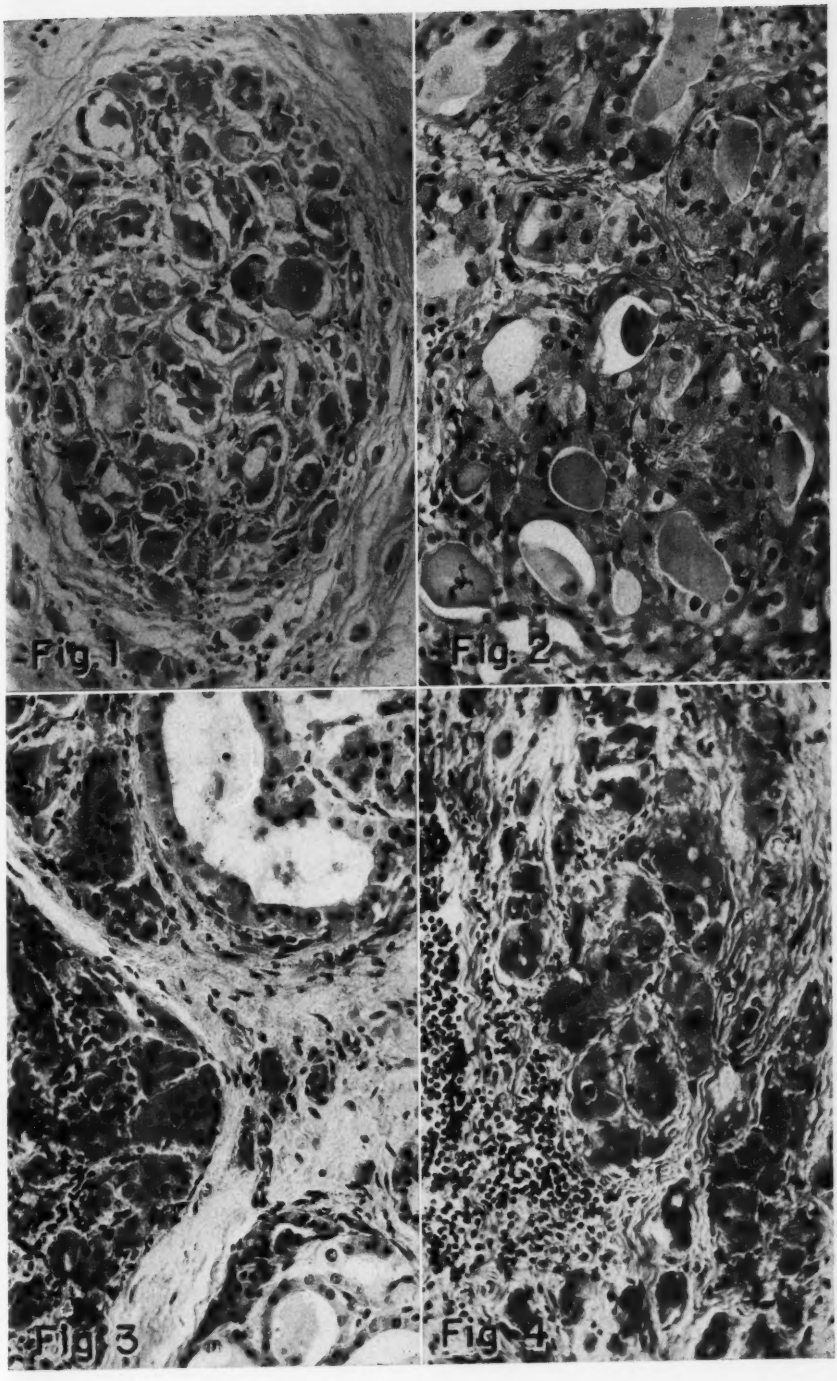

PLATE 1

volutionary alteration. In Hashimoto's disease the transition from cellular hyperplasia to cellular involution was evident. The belief expressed by Hellwig that Hürthle cells are " . . . young proliferating rather than degenerated and atrophic . . . " elements is based in part on his recognition that in the thyroid of Hashimoto's disease there is an intimate relation between Hürthle cells and hyperplastic elements. That the involution of hyperplastic epithelium is at times paralleled by functional alteration is evidenced by the old clinical observation that Hashimoto's disease and hypothyroidism may be sequelae of hyperthyroidism.

Certain types of supressive treatment increased the tendency to cellular involution, which is a consequence of prolonged stimulation. For example, Hürthle cells were often seen in simple hyperplasia; irradiation or treatment with thiouracil accelerated their formation. Similarly, in some instances in this series, continued proliferation of thyroid tissue despite multiple resections, resulted in cellular involution of the most extreme type.

Cellular involution is caused by prolonged hyperplasia without follicular involution (Fig. 13). It must be emphasized that such morphologically demonstrable hyperplasia is not necessarily associated with hyperfunction in the sense of excessive secretion. Deficiency of iodine or the administration of thiouracil, for example, result in hypofunction with concomitant cellular hyperplasia. Compensatory hyperplasia of thyroid epithelium ensues when there is failure of normal secretion. Either simple hyperplasia over a prolonged period or exaggerated hyperplasia, such as that caused by thiouracil, over a short period can result in cellular involution.

SUMMARY

By involution of the thyroid ("follicular involution") is meant that process in which follicular colloid is redeposited and follicular epithelium

FIG. 5. (Neg. No. 103335; Acc. No. 195181) Reaction to thiouracil. Focal transformation to Hürthle cells of hyperplastic thyroid epithelium. This patient had received thiouracil until ten days before resection was carried out. There were also many foci of atypical hyperplasia and pseudoneoplastic papillary conformations. (×185)

FIG. 6. (Neg. No. 103372; Acc. No. 168236) Reaction to thiouracil. Papillary and adenoid nodules composed almost exclusively of Hürthle cells in the thyroid of a man treated with thiouracil for exophthalmic goiter. (×23)

FIG. 7. (Neg. No. 103382; Acc. No. 198384) Reaction to partial thyroidectomy. Many nodules composed of Hürthle cells in a recurrent nodular goiter. The first operation had been performed eight years before (*see* Fig. 8). (×11)

FIG. 8. (Neg. No. 103337; Acc. No. 198384) Reaction to partial thyroidectomy. High power view of one of the nodules illustrated in Figure 7. It is composed entirely of dark Hürthle cells. (×185)

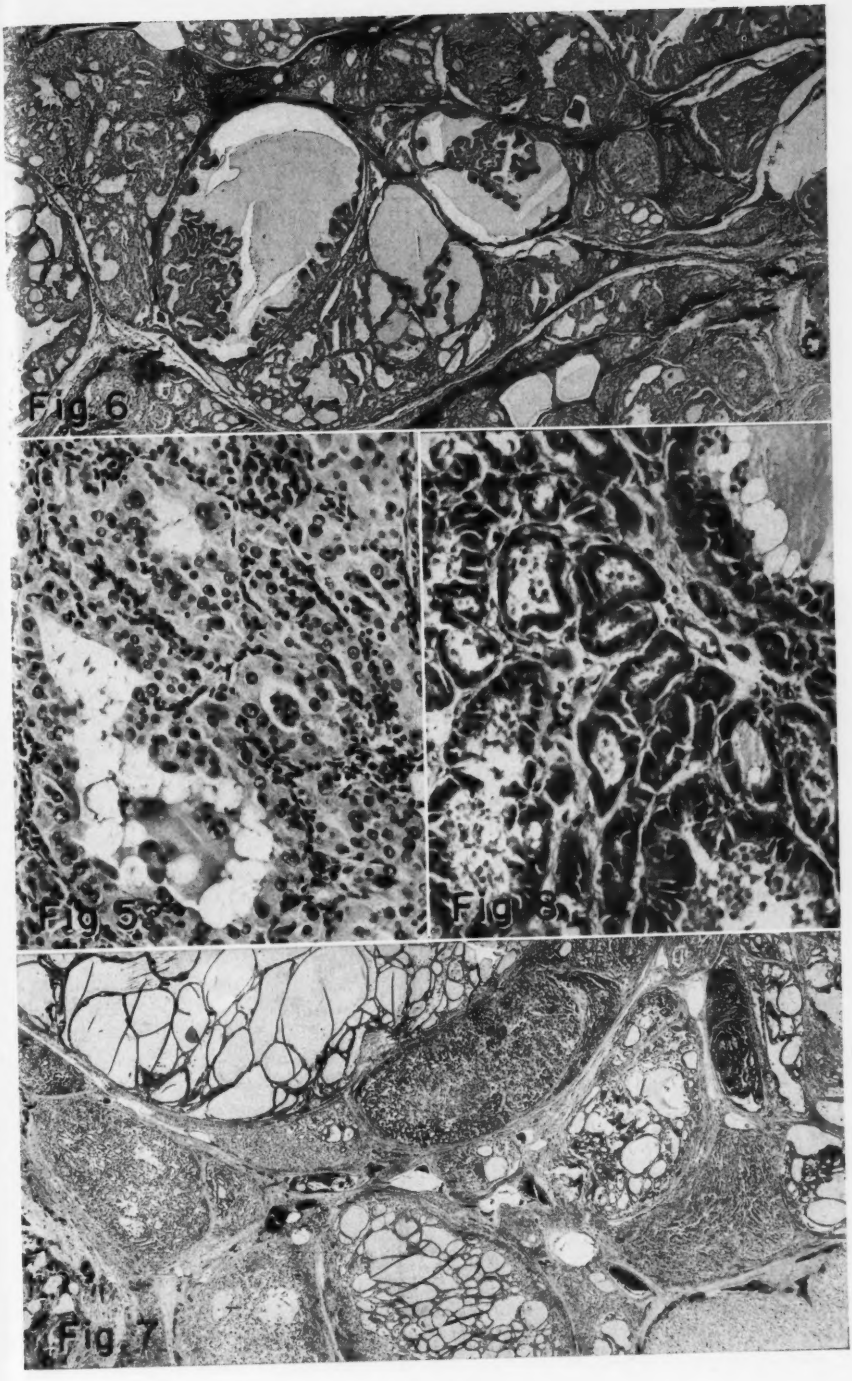

PLATE 2

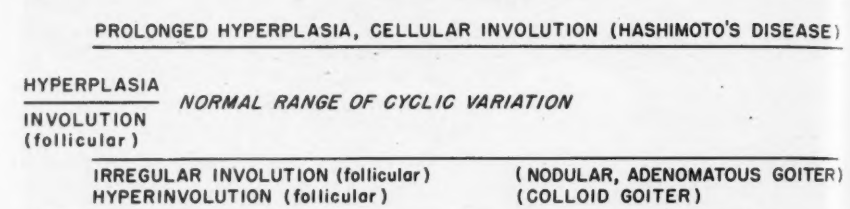

FIG. 13. Diagram indicating the difference in genesis of follicular and cellular forms of involution. The normal thyroid gland may undergo a recognizable degree of cyclic hyperplasia and follicular involution without resultant significant permanent structural alteration. Repeated cycles of excessive hyperplasia and follicular involution result in nodularity because proliferation and regression in different portions of the gland are not synchronized (3). Persistent follicular hyperinvolution leads to the development of colloid goiter.

returns to the flattened resting state after a period of hyperplasia. This phenomenon contrasts with certain other epithelial alterations which take place under a variety of conditions and might well be termed "cellular involution." The most prominent of these changes is the formation of the eosinophilic elements commonly called Hürthle cells in lesions of the thyroid generally regarded as regressive. Recognizing that cellular involution is common to a number of diseases may aid in the understanding of the pathologic physiology of the thyroid gland.

Numerous examples of cellular involution in the thyroid have been studied during the past five years at the Army Institute of Pathology. The conditions in which the phenomenon was observed included Hashimoto's disease, myxedema, "exhaustion" atrophy and exophthalmic goiter. It is of particular interest that identical alterations were observed consequent to roentgen therapy, treatment with thiouracil and subtotal resection. The occurrence of cellular involution can be taken as an indication that the

FIG. 9. (Neg. No. 103375; Acc. No. 184612) Reaction to partial thyroidectomy and thiouracil. Nodule of Hürthle cells in a gland which had twice been subjected to subtotal resection. The patient had also received thiouracil. (×55)

FIG. 10. (Neg. No. 103288; Acc. No. 86772) Reaction to irradiation and partial thyroidectomy. Focal development of Hürthle cells in a hyperplastic gland resected for the second time four years after irradiation and partial thyroidectomy, for exophthalmic goiter. Material removed at a third operation four years later revealed more diffuse and advanced cellular involution (*see* Fig. 11). (×185)

FIG. 11. (Neg. No. 103288A; Acc. No. 86772) Reaction to irradiation and partial thyroidectomy. This pattern was encountered in material removed at the third operation on the patient mentioned in the legend of Figure 10. There had been considerable regrowth of thyroid tissue. The small follicles composed of Hürthle cells and the lymphoid infiltration are identical with those seen in Hashimoto's disease (*see* Fig. 12). (×185)

FIG. 12. (Neg. No. 103294; Acc. No. 185263) Hashimoto's disease. Diffuse formation of Hürthle cells and lymphoid infiltration. Compare with Figure 11. (×185)

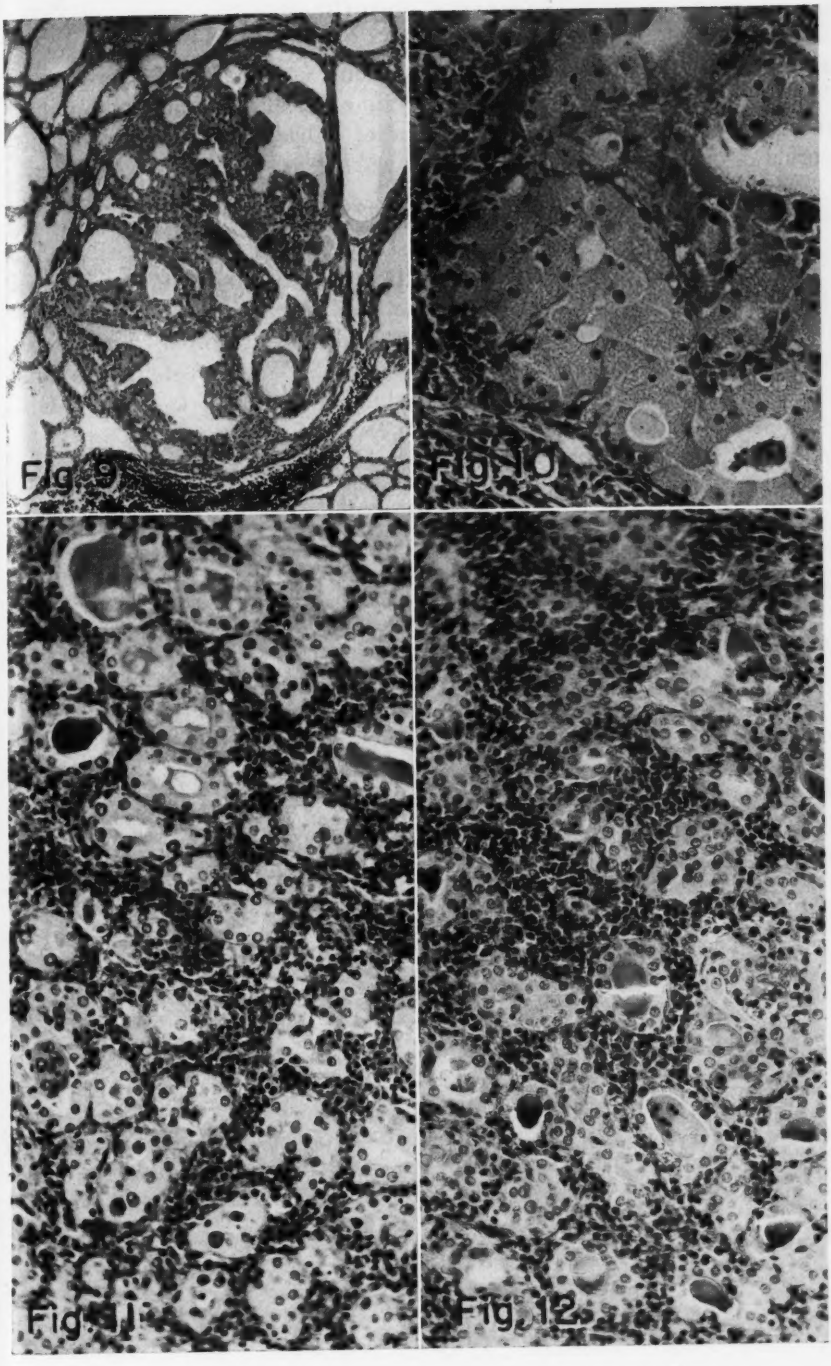

PLATE 3

altered glandular elements had "burned out" as a result of overstimulation. Thus the thyroid shares with certain other of the endocrine organs a tendency to enter an inactive phase after it has undergone a prolonged period of excessive activity.

Acknowledgment

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All of the photographs were taken by Mr. Roy Reeve.

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MYXEDEMA CIRCUMSCRIPTUM THYROTOXICUM

REPORT OF TWO CASES AND REMARKS ON ITS PATHOGENESIS AND TREATMENT

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INTRODUCTION

UNDER such names as "myxedema circumscriptum thyrotoxicum", "localized solid edema of the extremities in association with exophthalmic goiter" and "localized pretibial myxedema" are described cutaneous lesions of myxedematous appearance observed in patients with Graves-Basedow disease. Clinically these lesions are characterized by patches or cutaneous nodules, usually of very definite outlines, which as a general rule appear symmetrically on the anterior surface of both legs or on the dorsal surface of the feet (Figs. 1 and 2). Their size is variable; in some cases they are scarcely apparent, whereas in others they cover all of the distal segment of the inferior extremities. The overlying skin is erythematous, cyanotic or yellowish-white and the follicles are often dilated, giving an orange-skin or pig-skin appearance. Palpation reveals a swelling which does not pit on pressure (non-pitting edema), as well as hypothermia and the absence of perspiration. The lesions are always painless; some patients complain of mild itching and less frequently of heaviness in both legs.

The circumscribed myxedema is a cutaneous lesion with a chronic course. In most of the published reports the lesions were refractory to all therapeutic measures attempted; in some cases, after having remained unchanged for months or years there was spontaneous recovery.

It is a rare disease or at least it is rarely diagnosed. From the last decade of the nineteenth century in which Watson-Williams (1) and Morrow (2) described the first cases, up to the study published in 1946 by Cohen (3) in which the bibliography is brought up to date, only 78 cases have been reported. Among a series of 144 patients with various thyroid disorders studied by one of us in the course of the last year, we have observed 2 cases of circumscribed myxedema. The clinical histories of these 2 patients follow.

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FIG. 2. Showing the cutaneous lesions in case 2.

FIG. 1. Showing the cutaneous lesions in case 1. The scar of biopsy is still visible in the right leg.

CASE REPORTS

Case 1. Carmen J. was a 20-year-old girl from Lérida. Family and past history revealed nothing pertinent.

In December 1945 she showed nervousness, insomnia and asthenia. In a few weeks she lost 8 Kg. in weight. In February 1946 the presence of a diffuse goiter was observed and at the same time she complained of palpitation and diarrhea. The condition was diagnosed as hyperthyroidism and she was treated with homeopathic methods for three consecutive months, without improvement. In October of the same year, bilateral exophthalmos appeared and soon afterwards a striking improvement commenced. In September 1947 two indurated patches developed in both pretibial regions; they were clearly defined, of cyanotic appearance and slightly pruriginous. This cutaneous alteration developed during the course of about two weeks and then remained stationary.

On August 26, 1948, the basal metabolic rate was plus 32 per cent. The attending physician made a diagnosis of Graves-Basedow disease and prescribed 0.30 Gm. daily of methylthiouracil. This treatment was continued until October 28, when the patient was seen by us. With the antithyroid medication her nervousness, palpitation, fatigue and diarrhea improved considerably but the goiter, the exophthalmos and the cutaneous lesions were intensified.

Physical examination. Weight 63 Kg.; height 1.60 M. There was marked bilateral exophthalmos (Figs. 3 and 4) with slight edema of the eyelids (exophthalmometry RE = 22 mm., LE = 23 mm.). Gräfe, Möbius, Stellwag and Dalrymple signs were present There was a diffuse soft goiter with a vascular systolic thrill all over. The pulse was 160 and the blood pressure, 140/75 mm. Hg. The tendon reflexes were exaggerated.

Occupying the union of the medium third with the inferior third of both legs and situated on the antero-lateral surfaces, there were large patches, 15 cm. high by 10 cm. wide on the right extremity, and 20 cm. high by 12 cm. wide on the left. These patches were elevated by about 2 cm. from the surrounding skin. They were of a reddish-violet color, cyanotic, of a hard consistency, non-pitting, and perfectly delineated. Over their surface there was observed an adherent discrete desquamation, which when lightly scraped showed dilated follicles and a hypertrichosis. The hairs were not very numerous, were irregularly distributed, and were not found on the skin of the rest of the extremity (Fig. 1).

Both patches resembled the enormous blisters of Quincke's edema, from which, however, they were distinguished by their slow development, purple color, firmer consistency, the fine overlying desquamation and the hypertrichosis.

Biopsy of skin. (October 29, 1948). The epidermis conserved its normal structure, but the interpapillary prolongations were very strongly marked. The granular layer was hypertrophic and corresponded to the manifest hyperkeratosis throughout the skin, which was exaggerated still more at the level of the follicle orifices. The elective stains for melanin showed an increase of this element and its retention by the dermic chromatophorous cells.

In the papillary layer the elastic fibers were abnormal. The elastic fibrils had almost completely disappeared and the collagenous fibers had lost their fibrillar appearance. Some capillaries were observed surrounded by small cellular infiltrations that were chiefly lymphocytes.

The most marked lesions were seen in the corium. The fibrous layer of the skin was found to be completely divided by a clear edematous zone into two layers (Fig. 5), one superficial and the other deep, in which the pathology was practically the same. The

FIG. 4. Case 1. Lateral view of the exophthalmos and goiter.

FIG. 3. Case 1. Note marked exophthalmos and edema of eyelids.

collagenous network was dense but the appearance and coloring of the fibers tended to hyalinization. In certain zones of the microscopic preparation the interstitial lymphatic spaces were expanded and the fibroblasts showed ramification; this edema was only local and did not spread to the network or separate it. The elastic fibers, however, appeared fragmented, straight or in moniliform grains; some were well stained, whereas others had less affinity for the orcein and seemed about to disintegrate. From time to time there were visible at this level some perivascular infiltrations of mononuclear and lymphocytic cells. In the middle part of the corium the edema completely dominated the

FIG. 5

FIG. 6

FIG. 5. Case 1. The edematous zone which divides the corium into two layers is clearly distinguishable. This lesion appears characteristic of this disorder. Note mild hyperkeratosis of the epidermis and normal hypodermis (stain by the Masson's trichromic method).

FIG. 6. Case 1. Detail of the alterations of the elastic fibers in the deeper layer of the corium (stain by the orcein-hematin method).

picture and was so great that the space appeared almost empty (Fig. 6), partitioned only by an amorphous fibrillar wide reticulum which did not stain with thionin, mucicarmin or the acid and basic anilines. Scattered irregularly near this zone, there were a few bits of hyalinized collagenous substance of irregular outline or forming balls, which appeared thicker around some blood vessels which were sectioned at this level. The rare elastic fibers were distributed irregularly and presented more definitely the alterations

already described at other levels of the skin. Also around some vessels there were small lymphomonocytic infiltrations, more discrete than in the rest of the corium. In this edematous zone were found the pilosebaceous follicles. The fibers of the pili erector muscles appeared swollen and very refractive, whereas the sudoriparous glands were unaltered.

The hypodermis was normal, except for a panvascularitis affecting only the small arteries and veins feeding the skin.

Laboratory findings. The basal metabolic rate (October 29, 1948) was plus 12 per cent, blood cholesterol 145 mg. per cent, creatine retention 60 per cent, and urinary excretion of 17-ketosteroids, 10.7 mg. in twenty-four hours.

Thyrotropic hormone in the urine assayed 6.25 Junkmann-Schoeller units. Extraction was done by the Emerson and Cutting method (4); titration was done on the 24-hour-old chick by the Rawson and Salter microhistometric method (5).

Hematologic examination. Plasma and serum proteins and blood potassium, sodium, calcium and glucose levels were normal. The red and white blood cell counts were normal but there was a lymphomonocytosis.

Case 2. Maria de los Angeles T. was a single woman from Barcelona, aged 42 years. Family and past history were not pertinent.

The first signs of her illness began in September 1947 after an intense psychic trauma. She complained of heart pain, giddiness and palpitation. She was treated with digitalis during several months without relief. After January 1948, menstruation became less frequent and irregular. In March of the same year she observed bilateral exophthalmos which became progressively more intense.

On April 12, 1948, she was visited by one of us. She presented a very intense exophthalmos (exophthalmometry RE = 24 mm. LE = 23 mm.) with epiphora, palpebral edema and Gräfe, Möbius and Stellwag signs. Her thyroid gland was palpable. The heart rate was 130, with regular rhythm and the blood pressure was 150/70 mm. Hg.

Her basal metabolic rate was plus 44 per cent, and blood cholesterol 110 mg. per cent. Blood cell counts were normal, but there was a lymphocytosis.

With the diagnosis of Graves-Basedow disease, on April 13 treatment was started with 0.50 Gm. of thiouracil daily. On April 26 she was much better, the pulse having decreased to 90 and the metabolic rate to plus 26 per cent. The dose of thiouracil was reduced to 0.30 Gm. daily.

The initial improvement continued slowly and then the patient began to take thiouracil irregularly without any medical control.

On July 10 we saw the patient again with an obvious recurrence of thyrotoxicosis. Since the last fortnight in June she had noticed in both inferior extremities symmetrical cutaneous swellings distributed uniformly over the antero-lateral surfaces of the legs, commencing 3 cm. below the patella and ending 2 cm. above the metatarsophalangeal articulations (Fig. 2).

These swellings were solid, uniform, and did not pit on pressure. They were distributed in the form of a hemicylinder of which the continuity was broken at the level of the ankle by a deep groove shown in Figure 2. The color was a uniform reddish-purple. The cutaneous surface was covered by small funnel-like depressions corresponding to the pilosebaceous orifices, the abundance and uniformity of which gave to these patches the appearance of orange-skin.

On the cutaneous surface there was a fine adherent desquamation and an abundance of hairs, coarse, long and black, situated on the internal surface of both legs. The cutane-

ous alteration did not produce any discomfort, except a slight sensation of heaviness and tension.

The basal metabolic rate (July 10) was plus 34 per cent. Blood cholesterol was 125 mg. per cent. No permission for biopsy was obtained.

COMMENT

The cutaneous lesions observed in our 2 patients coincide completely with the published descriptions of "myxedema circumscriptum thyrotoxicum." Both patients presented typical Graves-Basedow disease; in the first patient cutaneous infiltration patches appeared during a phase of spontaneous partial remission of the thyrotoxicosis and coincided with the development of the exophthalmos; whereas in the second patient the "circumscribed myxedema" appeared at the beginning of a recurrence of the thyrotoxicosis after treatment with thiouracil

The pathogenesis of this disorder is a problem not yet solved. According to Sabrazes (6) these patches result from vasomotor alterations, causing blood and lymph stasis and chronic edema (*trophoedéme basedowien*). They are capable of resolving spontaneously or developing gradually to scleroderma.

O'Leary (7), Pillsbury and Stokes (8), and Ingram (9) believe that the lesions can be produced by chronic vascular stasis and cardiac failure secondary to the thyrotoxicosis. Traumatism has been considered as a possible causal factor of localization (Samek (10)). The action of cold could be one of the determining factors, according to Samek (10) and Carol (11), who point out the similarities of these alterations with erythrocyanosis crurum.

These hypotheses are not applicable in our cases nor in many others published by different authors in which the indurated patches appeared in mild thyrotoxicosis and even after the thyroid extirpation, without antecedent cardiac failure, traumatism, or the action of low temperatures.

There have been many endocrine hypotheses to explain the origin of these lesions. Richter (12) speaks of a state of thyroid exhaustion and considers that the disorder is produced by a functional alteration of antagonistic endocrine glands. Carol (11) believes that there exists in the legs a local hypothyroid state resembling the vascular disorders caused by cold. Urbach (13) and Wiener (14) think that the skin in the affected region has become incapable of using the thyroid hormone (either in normal quantities or in abundance), resulting in the local myxedematous alterations.

The "circumscribed myxedema" has been observed only in individuals who have or have had exophthalmic goiter. In some cases the patient showed definite thyrotoxicosis with a basal metabolic rate as high as plus

80 per cent (Goldner (15)), but in others it appeared in conjunction with a remission of the disease and with metabolic rates near the normal figure (cases of Samek (10), Urbach (13), Cohen (3)) or after thyroidectomy with marked hypometabolism (O'Leary (16), Netherton and Mulvey (17)). In the last type of case the administration of desiccated thyroid produces a rapid disappearance of the hypothyroid signs but the patches of circumscribed myxedema persist unmodified. In some patients the "circumscribed myxedema" develops either before or with the first manifestations of hyperthyroidism (O'Leary (7), Dowling (18)), whereas in other cases, such as our first patient, it appears when the hyperthyroidism has had a long period of evolution. The possibility of a tired thyroid gland might explain the last, but not the first situation.

The "thyrotoxic myxedema" in some cases starts with the formation of a soft diffuse edema, varying in intensity from one day to another and changing finally to the characteristic pretibial patches (O'Leary (16)). In other cases the lesions develop slowly and are delimited from the very first. Nodules have also been observed. These nodules are separate (Doessekker (19), Freudenthal and Brunauer (20)) but by confluence they reproduce the characteristic patches of the lesion (Dowling (18)).

The course of this cutaneous lesion is essentially chronic. In the published cases different hormonal and dermatologic treatments have been tested with practically negative results. Iodine, thyroidectomy and antithyroid drugs, desiccated thyroid and thyroxin, have been employed and shown to be useless or even capable of aggravating the process.

The histopathologic pattern of the cutaneous lesions also needs interpretation. According to the greater number of authors (*e.g.*, Pillsbury and Stokes (8), Netherton and Mulvey (17), Carol (11), Cohen (3)) the histologic picture is characteristic of hypothyroid myxedema. Dowling (18) and McLeod (21) speak of a mucinic degeneration of the corium secondary to a basophilic degeneration of the collagen and elastin, and Netherton and Mulvey believe that the lesion is completely different from genuine myxedema. They consider the condition to be a "mucoid degeneration of the skin" associated with hyperthyroidism. However, in our first observation some of the histochemical reactions for mucin were completely negative.

The conservation of the structure and the normal disposition of the papillae eliminates the possibility that these cutaneous lesions are due to a dermic edematous or other infiltration, which no doubt would have distended the epithelium, eliminating or at least decreasing the papillary festoon which was very prominent in our case. On the other hand, the situation of the lesion in the middle zone of the corium gives to the histologic picture a very specific topographic character and if we furthermore con-

sider the alteration of the collagen and elastic tissue which accompanies it, this infiltration could be interpreted as secondary to the alteration in elastic tissue produced by the endocrine disorder.

The presence of edematous alterations of the skin in patients with primary thyrotoxicosis is observed with relative frequency. The edema can affect the face, the extremities and specially the eyelids; it is transient and some times considered to be of angioneurotic type. It has no relation whatsoever to the intensity of the thyrotoxicosis. The exophthalmos, both that observed in Graves-Basedow disease and that which can be produced in laboratory animals by the administration of thyrotropic hormone, is due also to edema of the intra-orbital structures (Naffziger (22, 23), Naffziger and Jones (24), Jones and Naffziger (25), Aird (26), Smelser (27)); and, as happens in the other forms of edema and with the circumscribed myxedema, it is neither a constant sign of hyperthyroidism nor does it follow the course of the disease. In some cases the exophthalmos appears before any other thyrotoxic symptom (Klotz (28)); in others it manifests itself only in remissions or after the thyroid extirpation (Ruedemann (29)). In general, it increases after operation (Soley (30), Dobyns (31), and Dobyns and Haines (32)).

There exists then a series of analogies between the "circumscribed myxedema" and the exophthalmos. Exophthalmos is not due primarily to thyroid hyperfunction, as Gley (33) was able to produce it in the rabbit by means of thyroid extirpation and Friedgood (34, 35, 36) provoked it in the intact or thyroidectomized guinea pig by injection of pituitary extracts rich in thyrotropic hormone. Clinical and experimental studies have shown that the exophthalmos is a sign of hyperfunction of the anterior pituitary lobe produced through the thyrotropic hormone; or, as Friedgood (36) suggests, by mediation of an exophthalmotropic factor intimately related with this hormone.

"Circumscribed myxedema" is observed only in patients with intense exophthalmos and Cohen (3) refers to observations in which the ocular protrusion was so great that it should be classified as malignant exophthalmos. In such cases, in which the retrobulbar edema and the aqueous infiltration of the eyeball dominate the clinical picture, it is possible to demonstrate the urinary excretion of large quantities of thyrotropic factor, whereas in the hyperthyroidism not accompanied by exophthalmos and in normal individuals the excretion is very low or nonexistent (Rawson and Starr (37), Hertz, Williams and Means (38), Klotz (28, 39)). In our 2 patients there was a severe exophthalmos and in the first one we detected the excretion of a high titer of thyrotropic factor.

The parallelism between the exophthalmos and the "thyrotoxic myx-

edema" is accentuated even more if we consider the results of the different therapeutic methods which have been tested for each. We have already indicated that the exophthalmos is almost always accentuated after thyroid extirpation and may develop into dreaded malignant exophthalmos; "circumscribed myxedema" appears almost always in the remission phase of the disease and in many cases it only manifests itself after thyroidectomy. The antithyroid compounds such as thiouracil, possibly by their inhibitory action on the production of the thyroid hormone, in our patients produced an increase of exophthalmos and pretibial edema. Treatment aimed at inhibiting the anterior lobe of the pituitary gland (*e.g.*, estrogens, radiotherapy) gave good results in some cases of malignant exophthalmos studied by Friedgood (36), Brian (40), Martens (41) and Klotz (42). We therefore decided to test these procedures also in our cases of "thyrotoxic myxedema."

In the first of our patients we omitted the methylthiouracil and initiated treatment with 2 mg. daily of estradiol benzoate. After fifteen days the pulse rate had dropped from 160 to 80, the tremor of the fingers had disappeared and the weight had increased by 1 kilogram. From that date, the cutaneous patches gradually lost their cyanotic appearance, decreased in height and showed a tendency towards the formation of isolated nodules. The basal metabolic rate dropped from plus 12 to minus 18 per cent. After a month of treatment, a subtotal thyroidectomy was performed by Dr. Piulachs. After the operation the patient continued to take 2 mg. daily of estradiol benzoate in courses of treatment of three weeks' duration followed by a premenstrual interval of therapeutic rest. One month after the operation the metabolism was maintained at minus 17 per cent, the cutaneous patches had been considerably reduced, and the exophthalmos was very much less intense; urinary excretion of thyrotropic factor was not demonstrable.

In the second case the thiouracil was also omitted and treatment was begun with 0.5 mg. of diethylstilbestrol and 20 drops of Lugol's solution daily. The patient progressively improved and after seventeen days the basal metabolic rate dropped from plus 34 per cent to plus 12 per cent. The cutaneous infiltration, however, remained uninfluenced. To the prescribed treatment, 2 mg. of estradiol benzoate was added daily during intermenstrual cycles. After three months we again saw the patient. She had a small pretibial infiltration on the left leg, her exophthalmos had almost completely disappeared and she was free from hyperthyroid symptoms. Her basal metabolic rate was minus 7 per cent.

The action of thyrotropic hormone provides an explanation of the origin of the cutaneous infiltration patches which are observed in some patients with Graves-Basedow disease. Their elective localization on the pretibial

region of both legs possibly is due to the particular conditions under which the blood and lymphatic circulation is effected in this region.

SUMMARY

Two cases of "myxedema thyrotoxicum circumscriptum" are described. In one of the patients the lesions appeared after two years of Graves-Basedow disease; in the second, they developed during irregular treatment with thiouracil. In the first case the basal metabolic rate was plus 12 per cent; in the second, plus 34 per cent. Both patients were cured by the administration of estrogen in large doses.

The cutaneous biopsy showed in one case the presence of an edematous zone which divided the corium into two layers. This histologic lesion, with its specific topography and structure, seems to be characteristic of this disorder.

Circumscribed myxedema is a cutaneous lesion which, like malignant exophthalmos, is apparently caused by high production of pituitary thyrotropic hormone.

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GENERALIZED INSULIN ALLERGY

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INSULIN allergy is the term used to denote the specifically altered, acquired antigenic response to insulin protein in contradistinction to insulin sensitivity, which denotes an altered physiologic response to the hormone.

To illustrate this differentiation, Lowell (1), in extensive immunologic studies, presents evidence indicating the presence of two antibodies for crystalline insulin in one patient who was both allergic and resistant to crystalline insulin. He demonstrated the presence of a heat-labile allergic antibody which was capable of passively transferring skin sensitivity and an insulin-neutralizing antibody which was heat-stabile and capable of destroying the physiologic effect of crystalline insulin.

Various workers (2 a, b, c, d) have definitely established that crystalline insulin is a complete and soluble protein and, upon examining the insulins derived from the pancreases of various mammals (ox, sheep, hog) and fish, have found them to be similar in microscopic appearance, isoelectric point, solubility, and in carbon, hydrogen, nitrogen and sulphur content.

A considerable number of animal and human experiments have demonstrated the antigenic activity of insulin (3). Some authors (4 a, b, c) attempted to demonstrate the immunologic identity of the insulins derived from the pancreases of human beings, cattle, hogs, sheep, bison, and dogs. The results indicate that insulin lacked organ and/or species-specific factors. However, Lewis (5) in 1937, employing the Dale-Schultz uterine-strip technique, sensitized guinea pigs with beef or pork insulin and tested uterine strips with homologous or heterologous insulin and with beef pancreas and serum. He concluded that beef and pork insulin are closely related and have antigenic activity in common but are not completely identical, and he believes that there is a residuum of specificity in insulin which is inherent to the species but which differs from the other proteins of the same species.

Other factors have been implicated in the production of the allergic reac-

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tions. Animal protein or some other substance not removed from the pancreas in extraction may be present in the commercial insulin. Patients with positive reactions to skin tests with insulin obtained from various animal sources as well as with crystalline insulin have shown negative skin reactions with beef, pork, sheep, and horse proteins. These patients apparently manifest a true hypersensitivity to insulin which is not based upon a species-specific factor (6). On the other hand, it is a common clinical observation that patients who react unfavorably to insulin from one source may be able to tolerate insulin derived from another animal. Cohen and Simon (7) reported a case in which cutaneous tests definitely established this fact, indicating that the hypersensitivity was due to the animal protein source of the insulin and not to the pure insulin principle itself.

Insulin modifiers such as protamine have similarly been implicated as causative factors in allergic reactions to insulin. Cohen and Simon (7) state that certain individuals develop local reactions due to the protamine content of the preparation. However, Kern and Langner (8) performed intracutaneous tests with 0.02 cc. of protamine (salmine) solution on 100 control subjects and 104 diabetic persons who were receiving protamine zinc insulin (PZI). No positive reactions were obtained. All reports seem to indicate that positive cutaneous reactions to protamine insulin are always associated with reactions to other insulins such as beef, pork, and crystalline insulin, and not to protamine itself (6). Still other factors have been implicated such as the alcohol in which the syringe is kept, the preservative in the insulin, and the denaturing materials.

There are three types of reactions to insulin: 1) mild local reactions at the site of injection, 2) severe local reactions, and 3) general reactions. The mild local reactions at the site of injection usually consist of erythema or slight local induration and are probably nonimmunologic in nature and of little clinical significance. Severe local reactions usually consist of erythema and local edema, accompanied by pruritus. Such reactions may be immediate or delayed from six hours to several days after the injection. General reactions consist of generalized urticaria frequently associated with angioneurotic edema and with symptoms of circulatory failure, bronchial asthma, and gastro-intestinal symptoms.

The incidence of allergic reactions to insulin varies widely in the reports of different authors, apparently depending upon whether all local reactions as well as general reactions are reported. Hence such wide variations as Grafe's (9) 0.15 per cent, Collens, Lerner and Fialka's (10) 7.3 per cent, and Allan and Scherer's (11) 11.7 per cent have been reported. Allan and Scherer estimate that of persons hypersensitive to insulin, 98 to 99 per cent will show a local reaction and only 1 to 2 per cent a general reaction.

Thus, approximately one in a thousand will have a generalized reaction. Reviews of the case reports in the literature indicate that until recent years, only 63 definite cases with generalized reactions have been reported. At the last (1948) meeting of the American Diabetic Association in Chicago considerable discussion of this problem occurred with both allergists and diabetic specialists participating, and a plea was made that all cases of generalized reactions be reported in the literature so that a large enough series of cases could be accumulated to clarify many of the points in discussion.

At Hines Hospital, 2,949 diabetic patients have been admitted in the last eight years. We have treated just 2 cases of generalized reaction to insulin.

CASE REPORTS

Case 1: BAW, a 53-year-old white nurse first noted glycosuria during a routine urinalysis in 1928. A sister was also a diabetic. She stated that her diabetes had been controlled by diet alone and she had never taken insulin prior to her first admission to this hospital on January 10, 1938. Her blood sugar on admission was 181 mg. per cent. She was placed on a 1700 calorie (194 Gm. CH) diet and discharged eleven days later with a fasting blood sugar of 142 mg. per cent and was not given insulin. She was again admitted to the hospital on January 13, 1942, because of a furunculosis of the left popliteal region. On February 12, 1942, she was started on protamine zinc insulin, 16 units every morning, and on February 18, 1942, local pruritus was noted. On February 26, a 10 cm. reddened area at the site of the injected PZI was noted and the medication was changed to crystalline insulin, 16 units every morning. The difficulty subsided and following the clearing of the infectious process, her insulin requirements dropped and she was discharged on April 7, 1942, without insulin. She was again admitted in October 1942 for treatment of injuries suffered in an automobile accident, and the control of her diabetes appeared adequate without the use of insulin.

She was readmitted to this hospital on January 21, 1944, because of polyuria, polydipsia and severe neuritic pain in the extremities. Her fasting blood sugar was 200 mg. per cent. Because of the previous history of urticaria following insulin injections, she was cautiously started on crystalline zinc insulin, U.4-4-4 daily, on March 9. Ten days later a diffuse erythematous rash was noted on the face, neck and ears and on the following day giant urticaria appeared over the same areas and on the body and extremities as well. Insulin was stopped and the urticaria subsided in two days. However, her fasting blood sugars ranged between 275 and 375 mg. per cent and on May 1 she was given a test dose of 2 units of crystalline insulin. Within one hour giant urticaria with marked pruritus again appeared and gradually disappeared within the next few days. An attempt was then made to desensitize her to insulin. Three times daily she was given 1/5 unit crystalline zinc insulin well diluted with distilled water. This dose was doubled every three days until she could tolerate 13 units three times daily without reaction. She was discharged on June 30, 1944, taking 12–0–12 units daily with excellent control of her diabetes. She has had two subsequent readmissions to this hospital, the last in September 1946. Both were for the treatment of acute infections. She has been taking insulin regularly and has had no further allergic reactions.

Case 2: DJG, a 24-year-old white bus driver, was admitted to Hines Hospital on December 29, 1947, complaining of tender gums, progressive weakness, and weight loss of four weeks' duration. Prior to admission, he had been given a penicillin solution as a mouth wash by his dentist and oral medication by his physician without relief of symptoms. On admission his tonsils were enlarged, the pharynx was injected, and numerous small white ulcerations were seen on the buccal mucosa and gingival margins. A smear taken from the ulcerations showed Spirilla and fusiform bacilli. A single urine specimen showed 4 plus sugar and acetone. The initial blood sugar was 250 mg. per cent and the CO_2 combining power was 28 vols. per cent. Twenty-four hour quantitative urinalysis showed 75 Gm. of sugar in 3000 cc. of urine. His diabetic acidosis was readily controlled with intravenous fluids, crystalline insulin, and protamine zinc insulin. After five days of insulin therapy, he awoke on the morning of January 4, 1948, with extreme puffiness of the eyelids and lips and a generalized urticaria with intense pruritus. The protamine zinc insulin was discontinued and maintenance therapy on crystalline zinc insulin was attempted. However, his allergic manifestations grew worse despite use of antihistaminic drugs. Intradermal tests with beef and pork extract and protamine were negative but a 4 plus reaction was obtained with crystalline zinc insulin. The intracutaneous tests were repeated using amorphous regular insulin, crystalline zinc insulin, PZI, and globin insulin. There was a positive skin reaction to all these preparations.

On January 13 the patient was desensitized to PZI according to the rapid method described by Bayer (12), starting with 1/1000 unit intradermally and doubling the dose ever fifteen minutes until 1 unit was reached, followed by subcutaneous injection to the dose required for control of symptoms. Then daily injections of PZI were given. There was a mild generalized urticaria on January 15, but no symptoms at all after this. The patient was discharged on January 20, taking 24 units of PZI daily on a 2600 calorie diet.

He was readmitted on March 3, 1948, complaining of polyuria, polydipsia, polyphagia, weakness, fatiguability, and weight loss. He stated that since release from the hospital, his urine tests were negative for sugar and he had gradually decreased his insulin dosage, finally maintaining himself on diet alone and discontinuing insulin altogether. A few weeks later, glycosuria again appeared but on resuming insulin severe urticaria recurred and he was unable to take his insulin. The urine was strongly positive for sugar and acetone, blood sugar was 420 mg. per cent and CO_2 combining power was 44 vol. per cent. He was again desensitized to PZI according to the method of Bayer and concurrently was given pyribenzamine, 100 mg. four times. On this regime, occasional urticaria was noted for the first few days, which subsequently entirely disappeared. Protamine zinc insulin was started; subsequently crystalline zinc insulin was given twice daily as supplemental dosage, and he finally was given a 2:1 insulin mixture, which adequately controlled his diabetes. He was advised in the future to take a daily dose of insulin regardless of how well controlled his diabetes appeared to be.

DISCUSSION

Goldner and Ricketts (13), in 1942, stated that lapse of treatment seems to be important for the development of insulin allergy. They reviewed 15 cases reported in the literature and indicated that 9 of these cases had had previous periods of insulin treatment with intervals lasting from a few months to several years. They also stated that during the first course of treatment, the development of insulin allergy seems to be characterized

by a latent period varying from seven to twenty-five days, and they feel that the allergic predisposition apparently needs a period of sensitization for its activation.

Many methods of therapy have been advocated for the treatment of insulin allergy. The patient with mild local reactions often spontaneously desensitizes himself by simply persisting in the use of the kind of insulin to which he is sensitive. Other factors responsible for local reactions, such as chemical irritants and impurities in the manufacture of commercial insulin, have largely been eliminated by improved methods of extraction and purification. Mild local reactions can frequently be eliminated by injecting the insulin deeply into the subcutaneous or intramuscular tissues so that none gets into the skin. Harten and Walzer (6) feel that one of the most effective means of eliminating both local and general reactions is to change the brand of the insulin to one derived from another species or to employ crystalline insulin. There can be no doubt that changes in the preparation employed may overcome a nonspecific etiologic factor in some cases, or even a specific factor, such as sensitivity to the animal which is the source of the insulin. It is not likely to succeed in the presence of a true hypersensitivity to the insulin protein.

Specific desensitization has been used by numerous observers in a variety of methods. Allan and Scherer (11), Bayer (12), and Corcoran (14) have described methods of rapid desensitization in efforts to build up tolerance to insulin in one day. The Bayer method consists in intracutaneous injection, starting with 1/1000 unit and doubling the dose every 15 to 30 minutes as rapidly as the patient will tolerate it without allergic reaction, until a dose of 1 unit is reached. Subcutaneous doses can then be given as tolerated. Herold (15) reported a case in which desensitization was carried out over a period of three months. Another method is to give a very small dose of insulin one-half hour prior to the regular dose. Goldner and Ricketts (13) reported that in 6 out of their own 8 cases and in 11 of 15 cases reviewed in the literature, these procedures were unsuccessful and treatment had to be discontinued because of persistence or recurrence of allergic symptoms. Harten and Walzer (6) also point out that the loss of allergy to insulin may occur spontaneously and hence not all of the desensitizations reported in the literature can be accepted as authentic examples of the direct effects of specific therapy.

Nonspecific measures have also been advocated. Hunscheidt (16) suggested injection of calcium with the insulin. Strasser (17) administered calcium prior to the insulin. Collens, Lerner, and Fialka (10), and Roth and Rynearson (18), reported relief with subcutaneous injection of gradually increasing doses of histamine. Roth, Rynearson, and Horton (19) have re-

ported good results in eliminating local reactions by daily oral administration of 45 to 80 units of histaminase.

Leavitt and Gastineau (20) advocate the mixing of 0.5 to 1.0 cc. of a 1:1000 solution of diphenhydramine hydrochloride (benadryl) with the daily dose of insulin in the treatment of instances of local allergic reactions, reserving oral administration of the antihistaminic drug for the severe generalized reactions. Klein (21) reported the successful use of pyribenzamine hydrochloride in the treatment of a severe generalized reaction. He observed the interesting phenomenon that his patient was completely asymptomatic when given a daily morning injection of PZI and a noon injection of regular insulin covered by daily oral administration of 300 mg. of pyribenzamine. Discontinuance of the pyribenzamine resulted in an immediate recurrence of allergic symptoms. Then over a period of four weeks, the amount of pyribenzamine was gradually reduced until none was being used. With each reduction, there was noted local erythema, induration, and pruritus. Following this, the patient was asymptomatic for a month and then there was an occasional episode of local reaction which was controlled by a single daily dose of 25 to 50 mg. of pyribenzamine.

No immediately fatal reactions have been reported, although several deaths have been attributed indirectly to insulin allergy. Goldner and Ricketts (13), in commenting upon the discouraging results of treatment, state that it is a rather fortunate coincidence that the diabetes in most of these patients is of only moderate severity and with some effort can be controlled by diet alone. Although this is a comforting statement, it must be remembered that in the presence of infections, usually diabetic control is difficult to maintain without the use of insulin and if at such times insulin cannot be given the patient, there may readily be a fatal termination. As an example, our first patient following her desensitization had two subsequent admissions, both for rather severe acute infections. If it had been impossible to give her insulin at these times it is probable that she would not have survived the infection. The relative ease with which she was "desensitized" and the absolute lack of symptomatology subsequently makes us wonder whether or not she represents a case of spontaneous desensitization as described by Harten and Walzer (6) and whether her apparent response to the method of desensitization might have been merely coincidental. The second patient apparently represents an example of true hypersensitivity to insulin protein as evidenced by the skin tests as well as by the fact that upon desensitization to protamine zinc insulin, it was also possible to supplement his PZI with crystalline insulin without initiating an allergic response. It is interesting to note that prior to desensitization no benefit was obtained from the use of antihistaminic drugs; yet,

during his second course of treatment, only partial relief was obtained following desensitization, with excellent additional relief following the use of pyribenzamine. This patient also illustrates the important fact emphasized by Dr. Howard Root, that if one discovers a patient who is hypersensitive to insulin and manages to bring this difficulty under control, the patient should be advised to continue taking a small daily dose of insulin in order to maintain his desensitized state, even though his control appears adequate on diet alone. For, should the desensitization be allowed to lapse and the patient subsequently, for the usual variety of causes, develops diabetic acidosis, the one drug which would save his life, insulin, would not be available to him and might conceivably cause his death. We feel that since the advent of the antihistaminic drugs the outlook for these patients has immeasurably improved, since we can now combine one of the several methods of desensitization with the use of one of the antihistaminic drugs in order to control the symptoms during the acute phase and permit desensitization to be established. Once established, continued daily use of insulin would appear to be necessary in order to maintain the state of desensitization.

SUMMARY

1. The incidence of generalized allergy to insulin is small. Reports in the literature indicate that a generalized reaction develops in approximately 1 out of every 1,000 persons using insulin. In a large general hospital we have seen only 2 cases out of 2,949 admissions for the treatment of diabetes mellitus in the last eight years.

2. The importance is emphasized of attempting to desensitize such patients rather than relying on dietary control of the diabetes, since subsequent development of diabetic acidosis in the patient who is allergic to insulin may deprive him of the one therapeutic agent which could save his life.

3. The advent of antihistaminic drugs enables the physician to control more readily the patient's symptoms during the process of desensitization.

4. The importance of continued daily administration of insulin to prevent a lapse in the desensitized state is emphasized.

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THE GOITROGENS IN THYROTOXICOSIS COMPLICATING PREGNANCY

A CASE REPORT

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INTRODUCTION

THE administration of an antithyroid drug such as thiouracil or propylthiouracil during pregnancy must be undertaken with caution because of the potential hazards to the fetus. From theoretical considerations the danger is twofold: a) the drug may interfere with the embryonic development of the thyroid gland, altering its postnatal function permanently, or b) it may merely block synthesis of the thyroid hormone in-utero, leaving a gland capable of normal function after birth.

The thiourea derivatives pass the placental membrane in rats (1) and undoubtedly do so in humans. Davis and Forbes (2) found an enlarged, hyperplastic, thyroid in a 21-year-old mother who suddenly died during the sixth month of pregnancy while taking thiouracil.

A normal-appearing child at birth does not necessarily mean a normally functioning thyroid. Normal in-utero development occurs because of the maternal thyroid hormone which appears to diffuse across the placenta (1). Thus, the fetus does not suffer, even though its thyroid function is depressed by the goitrogen. The problem as to whether or not the embryonic development of the fetal thyroid has been altered so as to interfere with the postnatal function of the gland can only be answered by the observation of a sufficient number of children born of hyperthyroid mothers treated with goitrogens during pregnancy.

The occurrence of hyperthyroidism as a complication of pregnancy is rather rare. Mussey *et al.* (3) state that it occurs, on the average, in about 0.2 per cent of cases. Consequently, there have been but a few reports of hyperthyroid patients treated with the goitrogens during pregnancy. The following case is reported because it deals with a sixteen-month follow-up of a child born of a hyperthyroid mother who was treated with antithyroid drugs during pregnancy.

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CASE REPORT

M. E. (HH 4–188), a 27-year-old, white female was admitted to the hospital June 2, 1947, because of pain in the right lower quadrant of the abdomen and a weight loss of 40 pounds during the past six months. She was known to be about two and one-half months pregnant, her last menstrual period having occurred March 10, 1947. She had no symptoms of thyroid disease until one year before admission, when excessive nervousness and gross tremor of the hands was noted. There was a 40-pound weight loss (165 to 125 pounds) in spite of a good appetite. She always felt warm and used fewer covers than her husband.

Her past history revealed chorea at the age of 14, when she was hospitalized for three months. She was never told of any heart involvement at that time nor during the course of four pregnancies. Her menses were regular, occurring every twenty-eight to thirty days. She had four children; three were full term and one premature. All were living and well. Family history revealed that her mother had a goiter removed.

Physical examination: Pertinent physical findings revealed an excessively nervous, white female with marked psychomotor activity. Her temperature was 98.6 F., pulse 120, respirations 25, and B.P. 150/80. The skin was warm without any unusual perspiration. The eyes had a staring appearance with wide palpebral margins. The thyroid was diffusely enlarged with a marked thrill and bruit over both lobes. Examination of the heart revealed sinus tachycardia; no thrills or murmurs were present. There was mild tenderness in the right lower quadrant of the abdomen. Pelvic examination revealed a corpus enlarged to about a three-months pregnancy, with tenderness over the right adnexa. Marked tremor on extension of the fingers was noted.

Laboratory data: The hemogram showed red cells, 5,500,000; hemoglobin 14 Gm.; and white blood count 5,600, with a normal differential. The urine was normal, the serum cholesterol level was 179 mg. per cent, the B.M.R. was plus 70 per cent and the blood Kahn was negative. X-ray examination showed a normal chest.

Diagnosis: Diffuse thyroid hyperplasia with thyrotoxicosis and pregnancy.

The patient refused to remain in the hospital for further care and was discharged June 7, 1947, to be followed in the outpatient clinic. She was told to take thiouracil, 0.2 Gm. three times daily. Blood counts were done at each weekly visit and at no time did the white count fall below 4,800. The differential count was always within normal limits.

On July 17, 1947, her pulse was 116, and her medication was changed to 50 mg. of propylthiouracil three times daily. On August 15, 1947, her B.M.R. was plus 50 per cent and the electrocardiogram revealed a sinus tachycardia of 110. She was again seen November 28, 1947 (three days before delivery) and her pulse was 96, B.P. 145/80, and weight 156 pounds. The same dosage of propylthiouracil was continued. On December 1, 1947, spontaneous labor occurred and she was delivered of a normal, full-term, baby girl at another hospital. The delivery and puerperium were uneventful. The child was immediately given out for adoption according to a prearranged plan. The foster parents were made known to the writer, and the child has been followed for a period of sixteen months. During this interval the child has developed normally and presents no evidence of a hypothyroid state.

DISCUSSION

The normal birth and subsequent observation of a normally developing child for a sixteen-month period is in accord with the few previous reports of the administration of antithyroid drugs in pregnancy complicated by by hyperthyroidism (4, 5, 6, 7).

The comparatively long follow-up in this case shows that the child has a normally functioning thyroid and that neither thiouracil nor propylthiouracil altered the embryonic development and postnatal function of the gland. This fact can be established only by long term follow-up of such cases.

Chapman *et al.* (8) demonstrated that the fetal thyroid starts to take up radioactive iodine and probably begins to function by the fourteenth week. It may then be argued that in this case the antithyroid drug was not given until about the fourteenth week, when the fetal gland was already developed and beginning to function. Hence, the drug could not alter the embryonic development of the gland, but could merely suppress its in-utero function. Williams (4) has reported several cases in which the mother was treated before and during the entire pregnancy with thiouracil, without any harmful effect on the child. This observation supports the contention that the danger of these drugs to the fetus lies in suppression of the thyroid in-utero rather than in altering the embryonic development of the gland. The danger to the fetus appears to be from overdosage of the mother, resulting in the production of a hypothyroid state with insufficient thyroid hormone (maternal and fetal) to take care of the in-utero requirements (1).

Rune Frisk and Josefsson (9) reported a case of agitation, exophthalmos and low blood cholesterol in a child born of a hyperthyroid mother treated with methylthiouracil. This was attributed to an overactive anterior pituitary, caused by suppression of the fetal thyroid hormone by the drug. Although this was not proven, it suggests another possible danger in the use of these drugs during pregnancy.

SUMMARY

A case of thyrotoxicosis complicating pregnancy treated with thiouracil and propylthiouracil is reported. The child was followed for sixteen months and developed normally showing no ill effects from the drug. The potential hazards of the goitrogens to the fetus is discussed.

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ADDENDUM

Postpartum Course of Patient

The patient was next seen in the clinic on January 30, 1948. The nervousness and tremor had increased, even though she continued to take 50 mg. of propylthiouracil three times daily. She weighed 139 pounds, her pulse was regular with a rate of 120, and

Table 1. Résumé of clinical course of patient following second discharge from hospital

Date	Weight (pounds)	Pulse	WBC*	Propyl-thiouracil (mg.)	Remarks
5-21-48	152	84	6400	100 4 times daily	Bruit and thrill over left lobe. Lugol's solution, 5 drops 3 times daily.
6 -4-48	151		9900	100 4 times daily	
6-25-48	144	115		100 4 times daily	Bruit and thrill over both lobes.
7- 9-48	147		6800	100 4 times daily	Nervousness increasing.
8- 6-48	138	115	5800	100 5 times daily	
8-20-48	139	100	4100	100 5 times daily	Still taking Lugol's solution, 5 drops 3 times daily
9-10-48	138	104	4600		Still toxic.
9-17-48	141	128			Bruit and thrill over left lobe.
9-24-48	140	132		200 3 times daily	Serum cholesterol, 189 mg. per cent.
10- 1-48			4200	200 3 times daily	B.M.R. plus 95 per cent.
10-22-48	141	116	4800	200 3 times daily	Protein-bound blood iodine 13.3 micrograms per cent (normal, 5 to 7).
11- 5-48	140	116	5100	100 3 times daily	Bruit and thrill subsiding.
11-12-48	142	112		100 3 times daily	
11-26-48	142	110		100 3 times daily	Nervousness subsiding.
1- 7-49	135	120		100 4 times daily	
1-25-49			5100	100 4 times daily	B.M.R. plus 100 per cent.
2-18-49	138	115		100 4 times daily	Thyroid twice normal size.

* Differential always normal.

her B.P. was 145/80. The serum cholesterol was 200 mg. per cent, and the B.M.R. plus 44 per cent. Propylthiouracil was increased to 50 mg. four times daily. On March 19, 1948, she weighed 130 pounds, her pulse was 120, and her B.P. 145/80. The same dosage of the drug was continued.

(Second admission) The patient re-entered the hospital April 20, 1948 because of pain in the chest, back, and shoulders. On the day before, she had been seen in the admitting room because of the above complaints. Examination revealed auricular fibrillation with a rate of 120 and a pulse deficit. Hospitalization was advised, but she refused. Digitoxin was prescribed by the admitting room physician. She returned the next day because the chest pain became more severe after taking a total of 0.6 mg. of digitoxin.

Physical examination revealed her to be very apprehensive, and perspiring with a normal temperature and respiratory rate of 28. The staring of the eyes and widened palpebral margins were unchanged from the previous admission. The thyroid was diffusely enlarged with a loud bruit and thrill over both lobes. Examination of the heart revealed the apical impulse to be in the fifth interspace on the anterior axillary line. There was auricular fibrillation with a rate of 140 and an irregular pulse of 110. No thrills or murmurs were present; her B.P. was 140/80.

The hemogram was normal and the serum cholesterol level was 200 mg. per cent. An electrocardiogram revealed a flutter fibrillation with a rate of 140 and a left ventricular strain patter.

The patient was quieted with a sedative and given quinidine and 100 mg. of propylthiouracil three times daily. Conversion to a sinus rhythm occurred the following night. On the morning following conversion, an electrocardiogram showed a sinus rhythm of 100 and left ventricular strain. On April 23, 1948, the propylthiouracil was increased to 100 mg. four times daily. Her pulse varied between 80 and 100. Surgery was advised but she refused. She was discharged on May 11, 1948, taking 100 mg. of propylthiouracil four times daily and Lugol's solution, 5 drops three times daily. She was followed in the outpatient clinic. A résumé of her clinical course from the time of discharge to the present, is shown in Table 1. She has continued to take 400 mg. of propylthiouracil daily until the present time but the disease is still not under complete control.

Letter to the Editor

GLUCOSE TOLERANCE IN OSTEITIS DEFORMANS

To the Editor:

THE April 1949 issue of the Journal contains a letter by Dr. Robert C. Moehlig of Detroit advocating the administration of insulin for the relief of pain in patients with osteitis deformans. The rationale of this therapy was based on the finding of lowered sugar tolerance in the majority of patients with Paget's disease of bone.

We have investigated glucose tolerance in 11 patients with osteitis deformans by both the oral and intravenous tests and have failed to find any significant statistical difference between their glucose tolerance curves and those obtained from a control series of patients over 50 years of age. Both oral and intravenous glucose tolerance is depressed in older age

groups and, since osteitis deformans is a disease of middle and old age, studies using these tests must be compared to control series of normal subjects from similar age groups.

In other unpublished experiments, however, the influence of elevated serum alkaline phosphatase on carbohydrate metabolism in dogs was studied. The intravenous injection of a potent phosphatase solution prepared from intestinal mucosa produced serum alkaline phosphatase levels varying from 37 to 180 Bodansky units per 100 cc. with a gradual fall to normal levels in from one and one-half to three hours. Both oral and intravenous glucose tolerance tests performed during such a period of experimental hyperphosphatasemia were grossly elevated and prolonged as compared to control tests prior to the injection of phosphatase. The results of a typical experiment are as follows:

Control glucose tolerance test—0.5 mg. glucose per kilo intravenously in an 8-kilogram male dog.

Minutes	0	15	30	60	75	90
Blood sugar (mg. per 100 cc.)	92	145	106	88	83	90
Serum alkaline phosphatase (Bodansky units per 100 cc.)	9.6	—	—	—	—	—

Repeat glucose tolerance test after the intravenous injection of 3 cc. of phosphatase solution (200 Bodansky units per cc.)

Minutes	0	15	30	60	75	90
Blood sugar (mg. per 100 cc.)	93	328	234	168	137	—
Serum alkaline phosphatase (Bodansky units per 100 cc.)	8.0	91.4	73.9	24.1	17.5	—

The discrepancy between the clinical and experimental results remains unexplained. In several of our patients serum alkaline phosphatase levels were as high as those produced experimentally in dogs.

This brief presentation of our findings in Paget's disease does not, of course, alter the significance of the symptomatic response described by Moehlig and it is our purpose to investigate its effect in our own patients.

NORMAN G. SCHNEEBERG, M.D.
June 22, 1949.

1930 Chestnut Street,
Philadelphia 3, Pa.

The Association for the Study of Internal Secretions Request for Biographical Data for New Roster

The membership roll of the Association is being revised in preparation for the issuance of a new Roster as of December 31, 1949. *Copy for this must be in the hands of the printer not later than October 1, 1949.*

Return postal cards are being mailed to members requesting the following information:

Name..... Degrees.....

Mailing Address.....

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Teaching or Research Position:.....

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If in active practice of medicine, specialty:.....

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Members are urged to complete and return the card immediately and to keep the Secretary informed at all times of any change in the above.

HENRY H. TURNER, M.D.
Secretary-Treasurer
1200 North Walker Street
Oklahoma City 3, Oklahoma

The 1950 Meeting of the Association for the Study of Internal Secretions

The next Annual Meeting of the Association will be held in San Francisco, June 23 and 24, 1950. The hotel in which we shall meet has not yet been determined because of the uncertainty of AMA commitments, but it will be announced in an early issue of the Journal. Members are urged to make their hotel reservations as soon as possible.

The 1950 Awards of the Association for the Study of Internal Secretions

FELLOWSHIPS

The Ayerst, McKenna and Harrison Fellowship

The Ayerst, McKenna and Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin and in 1948 to Dr. Ernest M. Brown, Jr. Dr. Brown was re-elected for the Fellowship in 1949.

The Schering Fellowship in Endocrinology

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Lawrence Wilson.

Association Fellowships are designed to assist men or women of exceptional promise in their progress toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $2,500, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

AWARDS

The E. R. Squibb and Sons Award

The E. R. Squibb & Sons Award of $1,000 was established in 1939, and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945

to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; and in 1949 to Dr. Herbert M. Evans. In 1943 no award was given. A special committee of five members of the Association selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

The Ciba Award

The Ciba Award to recognize the meritorious accomplishment of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; and in 1949 to Dr. George Sayers. The Award is for $1,200. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $1,800.

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NOMINATIONS

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on *special application forms* which may be obtained from the *Secretary*, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 15, 1950.*

The 1950 Meeting of the American Goiter Association

The next meeting of the American Goiter Association will be held at the Hotel Shamrock, Houston, Texas, March 9, 10, and 11, 1950. It is recommended that all physicians wishing to attend make their hotel reservations early.

Award of the American Goiter Association

VAN METER PRIZE

The American Goiter Association again offers the Van Meter Prize Award of three hundred dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Houston, Texas, March 9, 10 and 11, 1950, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations, should not exceed three thousand words in length, and must be presented in English. A typewritten double spaced copy *in duplicate* should be sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East St. Vrain Street, Colorado Springs, Colorado, not later than *January 15, 1950.* The Committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award essay by the author, if it is possible for him to attend. The essay will be published in the annual Transactions of the Association.